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TWO SPECIES OF PYTHIUM OCCURRING IN SOUTHERN STATES

CHARLES DRECHSLER

(Accepted for publication July 18, 1942)

In several recent papers (19, 20, 21, 22) supplementary discussion and illustrations have been supplied for 11 of the 15 species of *Pythium* that I presented as new in diagnoses published 12 years ago (14). Similar treatment is accorded herein to *P. myriotylum,* one of the remaining 4 species, which occurs in some southern States as a parasite on economic plants. In addition, another fungus, likewise of southern origin, though not intimately akin to *P. myriotylum,* is described as a new member of the same genus. For facility of comparison all figures relating to the formation of oospores in both fungi are given at the same magnification (*i.e.,* × 1000) used for such illustrations in previous accounts; and similar magnification is employed for illustrations showing early stages in the germination of oospores. Owing to limitations of space, the figures pertaining to asexual reproduction by sporangia of mycelial origin are again given at half this magnification (*i.e.,* × 500), as are also the equally rangy figures showing zoosporangia produced in the later stages of oospore germination. The smaller scale of magnification applies no less advantageously to the drawings of the rangy branching clusters of appressoria in *P. myriotylum.* The elaborate intrication of hyphae, often observable when either of the 2 fungi is encountered by any one of several other oomycetes associated with root rot, also pertains to the vegetative stage; but as it entails frequently a complicated arrangement of parts, the larger scale of magnification has appeared generally preferable for the drawings relating to antagonistic or parasitic action in dual cultures.

PYTHIUM MYRIOTYLUM

In May, 1924, the late Dr. W. A. Orton, then in charge of investigations on truck crop diseases in the Bureau of Plant Industry, submitted to me a tomato (*Lycopersicon esculentum* Mill.) plant from South Carolina, with instructions to isolate from its discolored rootlets whatever parasitic fungi might be present in them. Among the several oomycetes obtained in pure culture from the affected material was a species of *Pythium,* which, except for its less massive development of sporangial lobules and its much readier production of zoospores in irrigated agar preparations, strongly resembled an allied form that I had often isolated from discolored roots of maize (*Zea mays* L.). The same species of *Pythium* was recognized the next month in a culture derived from a decaying cucumber (*Cucumis sativus* L.) fruit selected in Philadelphia as being illustrative of damage observed in a carload lot of cucumbers shipped from South Carolina. When the fungus was introduced by wound inoculation into sound marketable cucumbers, a rapid watery decay resulted, much like the "cottony leak" attributable to *P.*

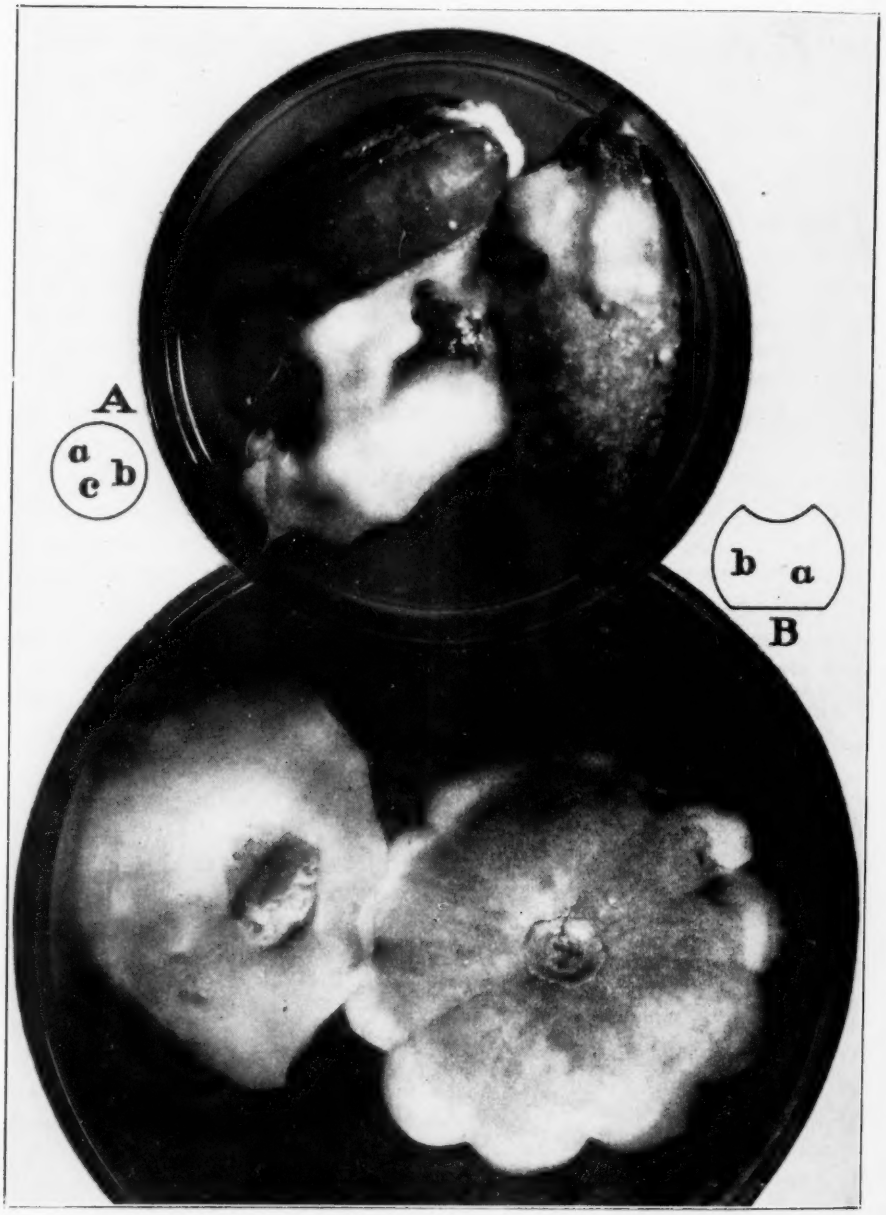

FIG. 1. A. Three cucumbers (a, b, c) infected with *Pythium myriotylum;* photographed 5 days after inoculation of specimen c was accomplished by placing on its uninjured epidermis a sizable slab taken from a young maize-meal-agar plate culture of the fungus, and then incubating in a damp chamber at 28° C. The parasite during the period of incubation spontaneously invaded the neighboring specimens a and b; about $\times \frac{2}{3}$. B. Two specimens of pattypan squash (a, b), each inoculated with *P. myriotylum* through an incision at the flower scar; extensive infection of the older and harder specimen (a) is shown only in a water-soaked area reaching nearly to the scalloped margin; the younger and tenderer specimen (b), invaded throughout, has become clothed in aerial mycelium; about $\times \frac{1}{2}$.

butleri Subr. For that matter, wounding was found unnecessary for successful infection. Under humid conditions and at fairly high temperatures (25 to 35° C.) mere application of sizable slabs of agar medium, newly permeated with mycelium, to uninjured epidermis consistently led to watery decay of the vegetable, and to its envelopment in abundant cottony mycelium (Fig. 1, A, a, b, c), which, on encountering adjacent cucumbers, caused their destruction in turn. Although this strong tendency toward aerial parasitism on cucumbers had been found alien to the maize-root fungus (9) its recognition as a physiological feature regularly associated with less pronounced sporangial lobulation and readier zoospore development required confirmation in additional cultures of separate origin.

Eleven such cultures, among a larger number referable to *Pythium butleri* and *P. acanthicum* Drechsl., were isolated early in June, 1925, from separate watermelon (*Citrullus vulgaris* Schrad.) fruits found partly destroyed by blossom-end rot in fields near Leesburg and Bradenton, Florida, and Thomasville, Georgia. A few weeks later 5 other cultures of the fungus under consideration were isolated from separate eggplant (*Solanum melongena* L.) fruits found damaged in fields near Bradenton by a ground rot closely resembling the "cottony leak" in the 18 similar specimens which yielded the cultures of *P. butleri* that, in a report (10) published at the time, were referred to *P. aphanidermatum* (Eds.) Fitzp. Under warm, humid conditions, introduction of the fungus into watermelon and eggplant fruits by wound inoculation always eventuated in watery decay of these vegetable products. The uninjured epidermis of both these fruits proved resistant to invasion, though in a few instances blossom-end infection, quite similar to spontaneous infection, resulted when a slab of agar medium newly permeated with mycelium was applied to the flower scar for 2 or 3 days. Eggplant fruits invaded by the fungus, whether from artificial inoculation or spontaneous infection, often afforded a copious growth of aerial mycelium (Fig. 2, A, B), but infected watermelons, with their hard rind of strongly indurated tissues, permitted no external development of the parasite. When slabs of agar medium, newly permeated with mycelium derived from any of the 16 cultures isolated from watermelon and eggplant fruits, were applied to uninjured cucumbers, watery decay promptly ensued, with abundant production of cottony aerial mycelium; and the aerial mycelium, on reaching other uninjured cucumbers, would closely invest them, penetrate into them, and thus destroy them in turn. In view of their capacity for aerial parasitism, as well as of their rather moderate sporangial lobulation and their ready production of zoospores in irrigated agar preparations, these 16 cultures, while generally resembling the maize parasite, differed from it in the same particulars as the 2 cultures previously isolated from material originating in South Carolina. The 18 cultures, therefore, were considered to belong to a separate species, which, in 1930, I described under the binomial *P. myriotylum*, 2 years after I had presented the maize parasite as *P. arrhenomanes* (12).

Thus at the time of its description *Pythium myriotylum* had become known to me as a root-rotting parasite only through a single encounter. Later, in 1931, I recognized the species in a number of cultures received from S. C. J. Jochems of the Deli Proefstation at Medan, Sumatra, where they had been isolated in connection with studies on a serious foot rot most

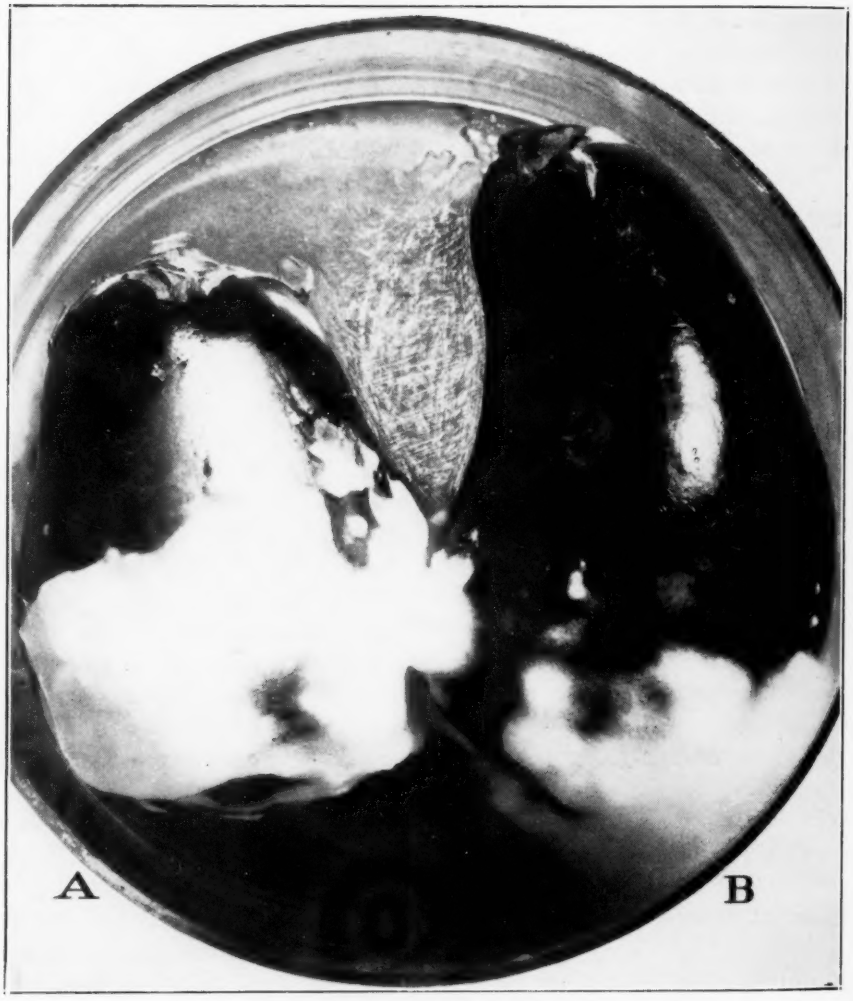

FIG. 2. Two eggplant fruits (A and B) infected with *Pythium myriotylum;* photographed 5 days after each had been inoculated by introducing the fungus into an incision at the stylar scar; incubated in a damp chamber at 28° C.; about × ½.

often attacking tobacco (*Nicotiana tabacum* L.) seedlings newly transplanted from seedbed to field. In an extended account, published in 1927, Jochems (25) had given a full description of the disease in question, denominating it as "parasitaire Stengelverbranding" or "parasitic stem-burn," and had indicated as causal organisms 4 unidentified species of

Pythium, which he designated provisionally by the letters A, B, C, D, though holding that *P. debaryanum* Hesse and *P. aphanidermatum* were probably included among them. Somewhat earlier, to be sure, in a list of plant diseases and pests attacking cultivated plants in the Dutch East Indies (23), responsibility for causation of "Stengelverbranding" was attributed to species of *Pythium* cited under the 4 binomials *P. debaryanum*, *P. butleri*, *P. polyandrum*, and *P. nicotianae*. Descriptions applicable to the latter 2 binomials have never been supplied. However, in 1934, Meurs (27) made known that the fungus cited by van Hall (23) in 1925 as *P. polyandrum* was the same as *P. myriotylum*, which species, together with 2 congeneric forms, he had discovered in scores of cultures isolated by him from diseased tissues of tobacco plants found affected with stemburn in Sumatra during the period from 1929 to 1932. With respect to the nomenclature of the parasite, Meurs dismissed van Hall's binomial from consideration as a *nomen nudum*. In the meantime, incidentally, the specific component of that binomial—or rather an orthographic variant not considered different according to established rules of botanical nomenclature (3, p. 22, Art. 70, Note 4)—had been applied by Sideris (32) to a closely related and unquestionably congeneric fungus which he described as new under the binomial *Nematosporangium polyandron*, but which Rands and Dopp (30) have subsequently found referable to *P. arrhenomanes*.

Further indication that *Pythium myriotylum* occurs in affected vegetative parts of crop plants was provided when the fungus came to light in one of 3 pure cultures submitted to me for identification in March, 1940, by Dr. M. N. Walker of the Watermelon and Ornamental Field Laboratory at Leesburg, Fla., where they had been isolated from watermelon seedlings (35). Recently, too, the parasite has been reported unambiguously by additional observers in foreign lands. In 1936 Park (29) noted its occurrence as the cause of a soft rot affecting ginger (*Zingiber officinale* Rosc.) imported from India and planted in Ceylon. Subsequently Uppal (33) stated that the fungus causing soft rot of ginger in Surat had been determined to be *P. myriotylum*. According to Wager (34) *P. myriotylum*, together with 2 congeneric forms, was isolated once from papaw (*Carica papaya* L.) plants affected with "foot rot" in South Africa. Though a species of *Pythium*, isolated in Sierra Leone from garden bean (*Phaseolus vulgaris* L.) plants affected with wilt, was determined only rather indefinitely as being near *P. myriotylum*, Deighton's account (7) of the manner in which under moist conditions its woolly mycelium soon covered the stems of infected plants and then spread over the ground to attack other seedlings in its path, very aptly describes the growth habit of *P. myriotylum*.

That *Pythium myriotylum* may really have been the species encountered by Deighton appears the more likely from its aggressive parasitism on snap beans under experimental conditions (Fig. 3, A, B). Such parasitism on the uninjured immature edible pods of garden beans was reported in 1927 by Harter and Whitney (24), who found the fungus capable of bringing

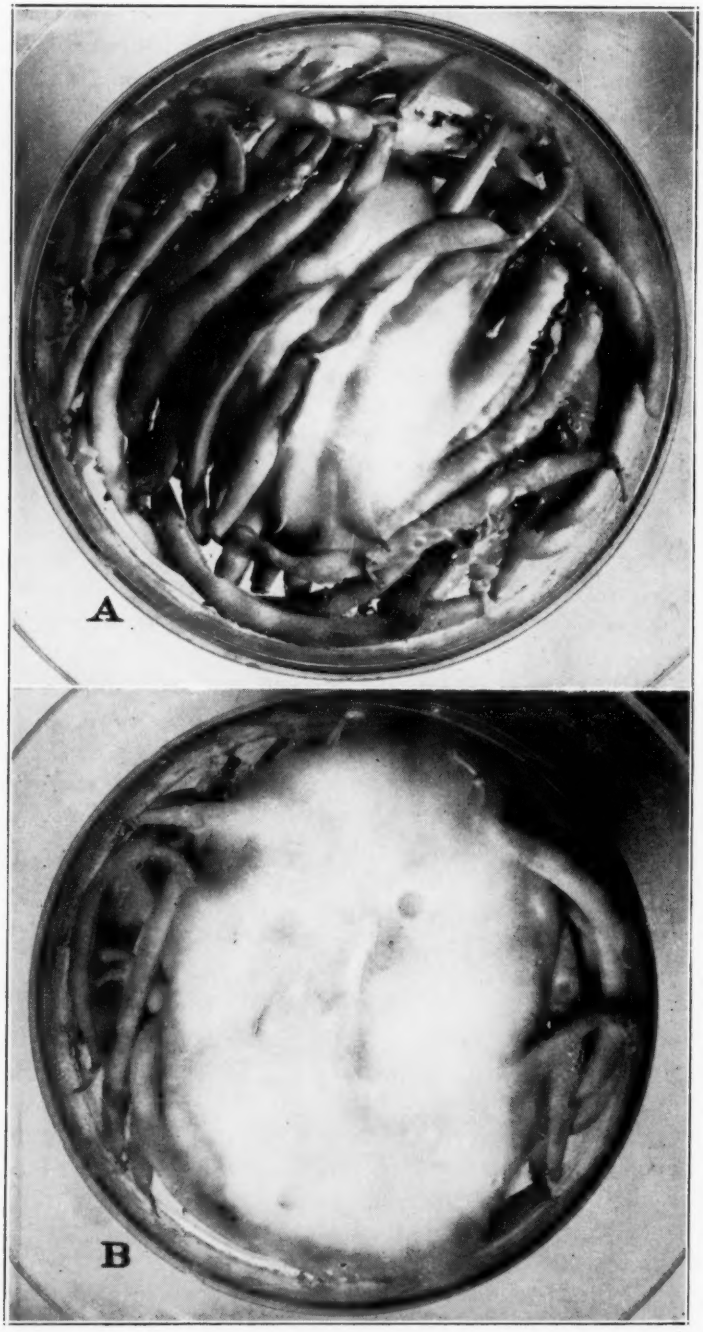

FIG. 3. String beans showing progressive attack by *Pythium myriotylum*, after a sizable slab of maize-meal-agar medium, permeated with mycelium, had been placed on the uninjured pods in the center of the dish. A. Photographed after incubation at 28° C. for 3 days. B. Same material photographed 2 days later, that is, after incubation at 28° for 5 days; about × ½.

about a decay of this vegetable product very similar to the "nesting" caused spontaneously in transit by the congeneric *P. butleri,* designated by them as *P. aphanidermatum.* Like cottony leak of cucumbers, the decay of bulked snap beans caused by *P. myriotylum* is accompanied by production of profuse cottony mycelium. Profuse aerial mycelium is usually produced also in the destruction of summer squashes (*Cucurbita pepo* L.) under moist conditions, following artificial inoculation with the fungus; the larger, older, and somewhat harder specimens of this vegetable (Fig. 1, B, a) permitting such production less promptly than younger and tenderer specimens (Fig. 1, B, b). Wound inoculation of the parasite into muskmelons (*Cucumis melo* L.) and Cassaba melons (*C. melo* var. *inodorus* Naudin) ordinarily leads to no conspicuous cottony development externally, though the flesh within the hard rind often becomes extensively permeated with mycelium, and, though aerial mycelium often is produced within the central cavity.

In pure culture, especially at rather high temperatures, *Pythium myriotylum* extends its mycelium more rapidly than most allied forms. Accordingly, it must be reckoned, together with such familiar pathogenic species as *P. ultimum* and *P. butleri,* among the fastest-growing of fungi. Further similarity to the 2 species mentioned is apparent not only in the somewhat coarse texture of its mycelium, but also in the disorderly arrangement and promiscuous ramification of its mycelial filaments. On maize-meal-agar media, or on other media not rich in nutrients, aerial mycelium may be produced in only small quantity, particularly if the overlying or surrounding air is deficient in moisture. However, if a humid atmosphere is maintained in a Petri dish containing a plate culture prepared with a medium rich in nutrients as, for example, Lima-bean agar, the dish often becomes completely filled with aerial mycelium of such density and firmness that the basal part of the container may be held up loosely suspended from the lid.

Microscopic examination of the under side of a Petri-dish culture prepared with maize-meal agar, usually shows everywhere a generous scattering of knob-like appressoria in contact with the glass floor. When thus produced at the interface of agar medium and glass, these organs commonly measure about 7 or 8 μ in diameter, and consist of swollen terminations on rather short lateral branches, though their development as lateral protuberances on longer hyphal elements is not infrequent (Fig. 4, A, B). With respect to the abundance of such intramatrical appressoria, *P. myriotylum* appears comparable to *P. butleri* and other strongly parasitic members of the genus. However, with respect to the abundance of its production of appressoria on the surface of solid objects encountered by its aerial mycelium, the fungus is unrivalled by any congeneric form known to me. In Petri-dish cultures with copious aerial development the ceiling of the container often is beset everywhere with dense clusters of appressoria borne on systems of closely ramifying branches (Fig. 4, C, D). The adhesive organs formed here usually measure from 8 to 11 μ in diameter, and thus are notice-

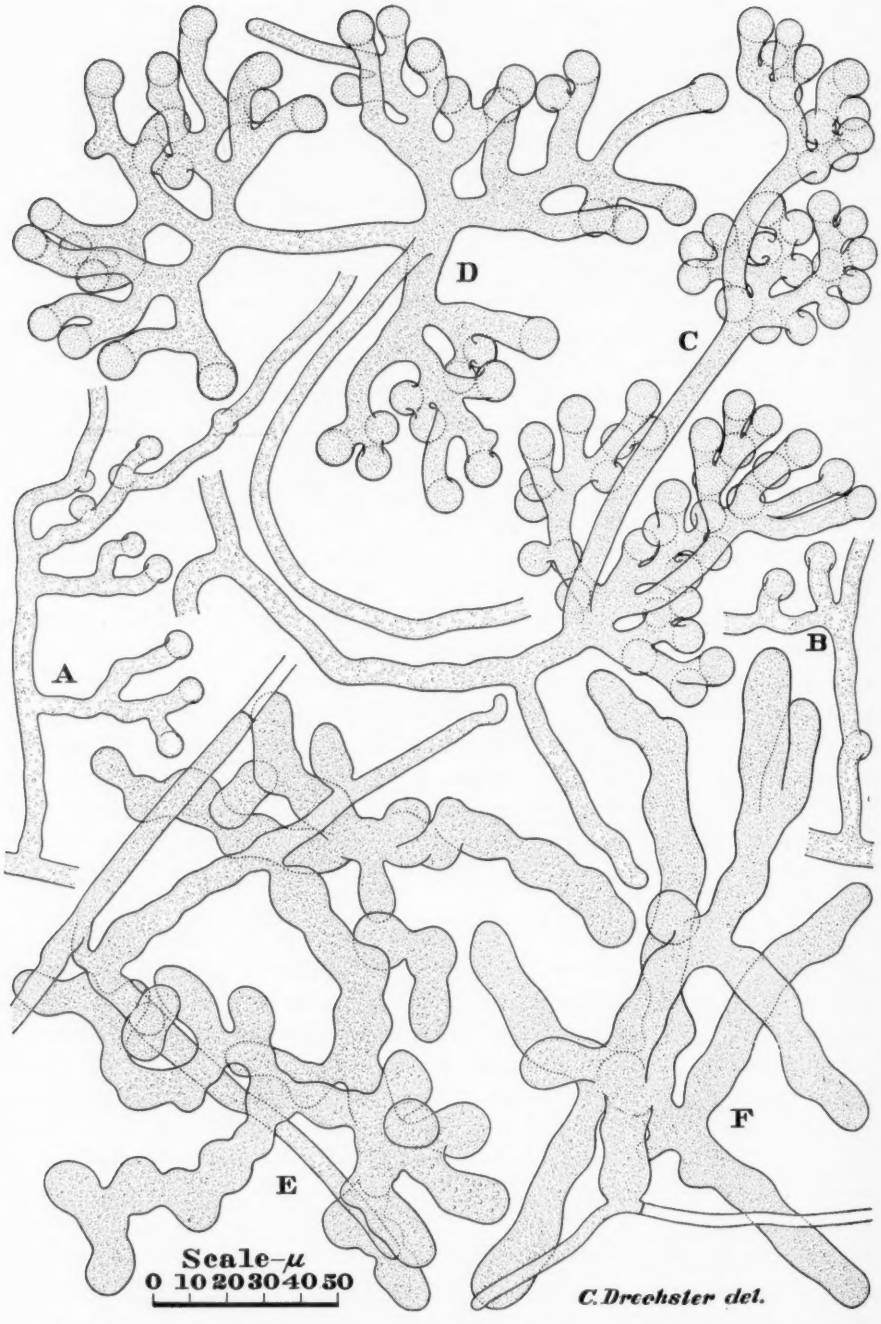

FIG. 4. Vegetative and asexual reproductive structures of *Pythium myriotylum* produced in Petri-dish cultures; drawn to a uniform magnification with the aid of a camera lucida; ×500 throughout. A, B. Portions of submerged mycelium bearing appressoria in contact with floor of container. C, D. Portions of aerial mycelium bearing appressoria in contact with ceiling of container. E, F. Systems of swollen sporangial branches formed on surface of maize-meal-agar medium.

ably larger than the homologous organs produced under the medium. Unquestionably the abundant development of aerial appressoria by the fungus is related to its capacity to operate as an aerial parasite in the causation of such trouble in bulked vegetables as cottony leak or nesting.

When grown in plate cultures on maize-meal agar or Lima-bean agar, *Pythium myriotylum* often produces, especially in areas where the surface of the medium is moist with water of condensation, more or less branched systems of swollen digitate elements (Fig. 4, E, F; Fig. 5, A). Like the homologous, though usually more extensive, ramifying complexes produced under similar conditions by *P. butleri* and *P. complens* Fischer (=*P. torulosum* Coker and Patterson), these branched systems, on being bathed in fresh water, give rise to evacuation tubes and undergo transformation into zoosporangia; the smaller systems usually being converted into single zoosporangia, the more extensive ones into plural zoosporangia. Development of swollen branches takes place in some measure also when young undifferentiated mycelium is supplied with fresh water under conditions suitable for prompt production of zoospores; noticeably swollen elements being formed when, for example, small pieces of a newly invaded watermelon or cucumber, or small slabs excised from a young Lima-bean agar culture, are transferred to a shallow layer of water. Though some of the sporangia partitioned off as separate reproductive units in such a preparation may include perhaps a dozen swollen branches (Fig. 5, B) together with hyphal parts of smaller volume and an evacuation tube (Fig. 5, B, t), many other sporangia may consist of a rather long portion of unmodified filament together with only a few swollen branches (Fig. 5, C, D). Indeed, many of the smaller sporangia may include no perceptibly swollen elements whatever (Fig. 5, E). Although an evacuation tube arising from a swollen part (Fig. 5, B, t; F, t), or one arising laterally from a rather wide undifferentiated hyphal part (Fig. 5, E, t), is usually recognizable as a special structure, similar distinctiveness is ordinarily absent when an evacuation tube is formed through prolongation of an outwardly unmodified branch or filament (Fig. 5, C, D). Functional frustration of evacuation tubes seems rather more frequent here than in most congeneric forms; the frustrated tube soon being walled off in whole or in part by deposition of a septum (Fig. 5, F, a), and a new evacuation tube thereupon being put forth from some other position (Fig. 5, F, t).

Zoospore formation in *Pythium myriotylum* is associated with the sequence of internal changes usual for members of the genus. As the evacuation tube attains definitive length, and forms a hyaline cap of dehiscence (Fig. 5, B, t), the protoplasmic contents of the sporangium show increasing vacuolization. The hyaline cap suddenly yields to become inflated into a vesicular membrane as the migrating sporangial contents accumulate within it. Once the migration is completed the undifferentiated granular mass (Fig. 5, C; F, t) undergoes division, and in the course of about 20 or 25 minutes is transformed into laterally biciliate, motile zoospores (Fig. 5, D,

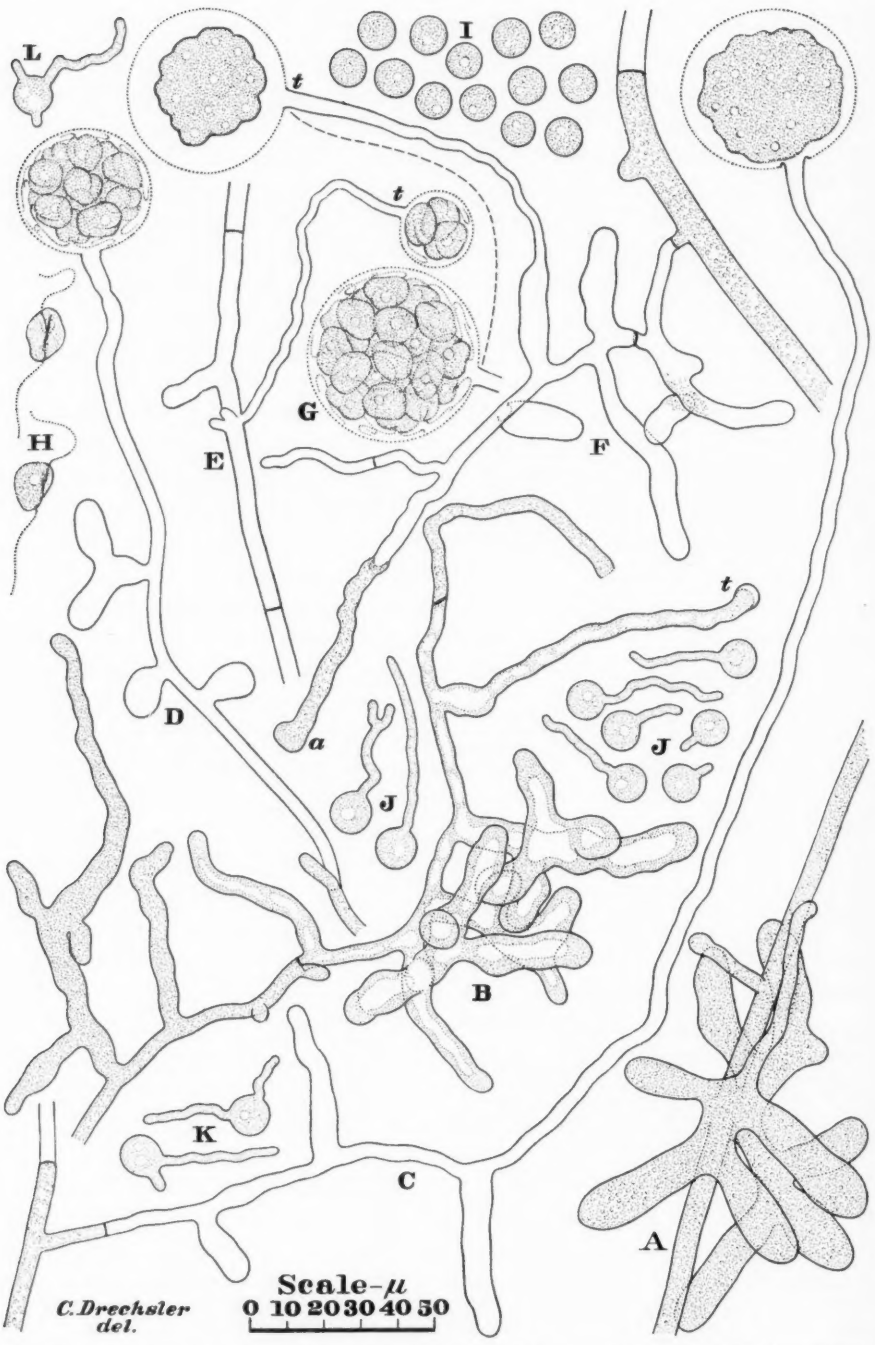

FIG. 5. Asexual reproductive apparatus of *Pythium myriotylum*; ×500 throughout. A. Hypha bearing distended sporangial branches. B. Vacuolate sporangium shortly before discharge. C. Sporangium shortly after discharge. D, E. Empty sporangia with their zoospores nearly ready to escape. F. Sporangium that, after frustration of its first evacuation tube (a), has formed a vesicle at the tip of a second tube (t). G. Same vesicle at a later stage. H. Motile zoospores. I. Encysted zoospores. J, K, L. Germinating zoospores. (t, evacuation tube.)

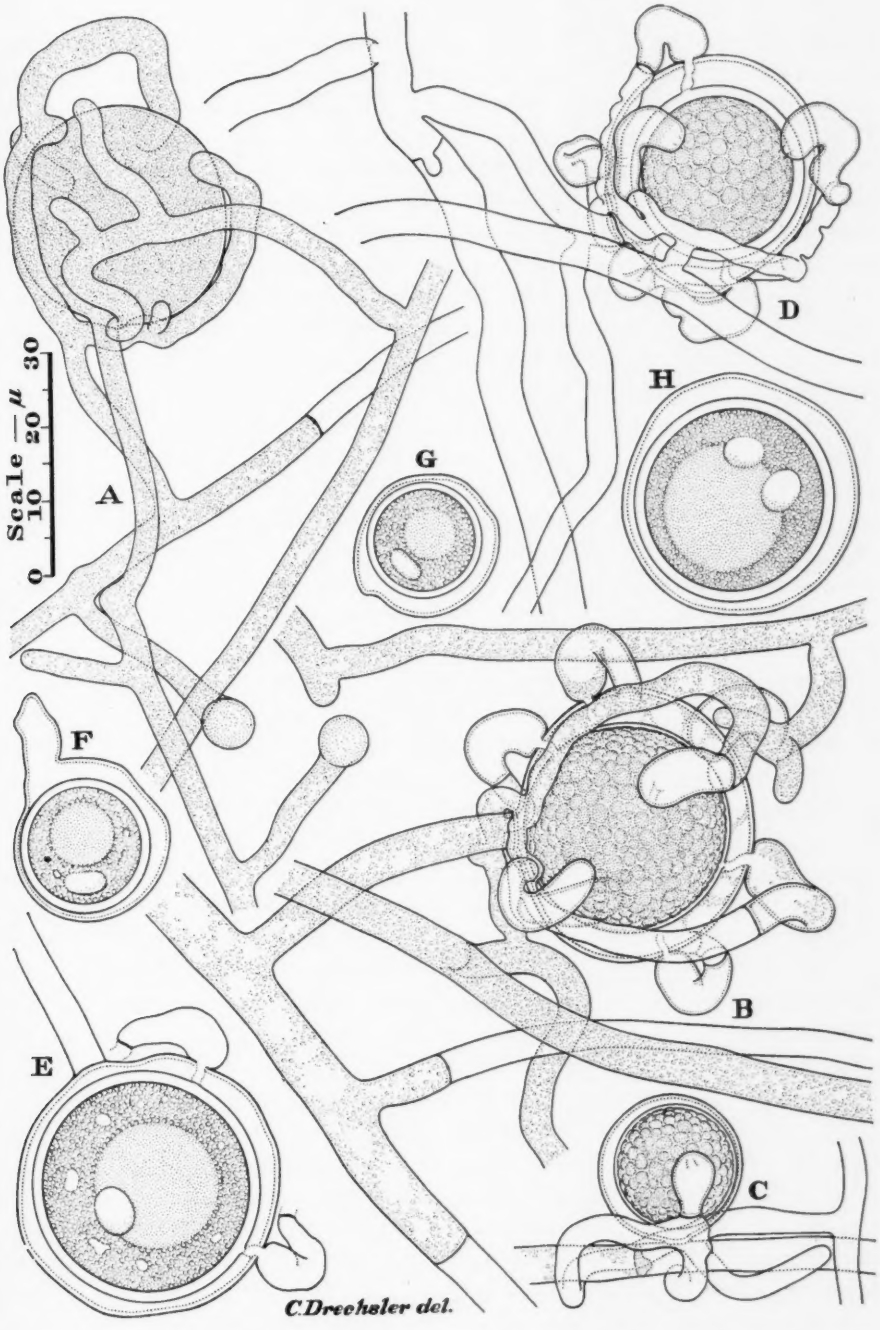

FIG. 6. Sexual reproductive apparatus of *Pythium myriotylum* formed in maize-meal-agar cultures; drawn to a uniform magnification with aid of a camera lucida; ×1000 throughout. A–D. Immature units showing antheridial relationships. E. Large oogonium with mature oospore; showing two antheridia in profile view. F–H. Oogonia, each with a mature oospore.

E, G). After increasingly violent activity the zoospores escape from the disintegrating vesicle and swim about for some time (Fig. 5, H) to round up into subspherical cysts (Fig. 5, I), which sooner or later germinate by the production of a single germ tube (Fig. 5, J), or of 2 (Fig. 5, K) or even 3 (Fig. 5, L) germ tubes.

Pythium myriotylum, whether growing in the tissues of host plants or in various artificial agar media, usually shows fairly abundant sexual reproduction. The earlier stages in the enlargement of the subspherical oogonium ordinarily precede development of any accompanying antheridia, though, as a rule, antheridial branches are found wrapped extensively about the expanding female organ before it attains definitive size (Fig. 6, A). The enveloping branches soon give rise terminally or, less often, somewhat laterally, to crook-necked male organs, each delimited proximally by a cross-wall. At nearly the same time the oogonium, having completed its growth, likewise is delimited from its supporting hypha. Contraction of the oogonial contents into a spherical body of lumpy structure (Fig. 6, B, C, D; Fig. 7, A, B, C) ensues, with intrusion of fertilization tubes, and movement of antheridial materials through them. The spherical body, after laying down a thick wall, undergoes internal reorganization whereby it is converted into a yellowish oospore that at early maturity (Fig. 6, E, F, G; Fig. 7, D) reveals the unitary structure most usual in oospores of the genus,—its single reserve globule being surrounded by a parietal layer of granular protoplasm, in which a single subspherical or oblate ellipsoidal refringent body is imbedded. After several weeks of further ripening, some oospores will frequently be found to contain 2 refringent bodies (Fig. 6, H; Fig. 7, E); and additional increase in number of these small but conspicuous structural elements may come with more prolonged aging. With extended aging, too, the parietal layer often acquires a more coarsely granular texture almost like the texture of the homologous layer in species of *Aphanomyces.*

The sexual apparatus of *Pythium myriotylum* shows much variety with respect to the origin and positional relationships of its component parts. Most often, perhaps, the oogonia are borne terminally on branches of variable lengths (Fig. 6, A, B, D; Fig. 7, A, B, C), yet their occurrence in lateral (Fig. 6, C; Fig. 7, E) or intercalary (Fig. 7, D) positions is not infrequent. The male complement may be supplied wholly from a single parent filament (Fig. 6, D; Fig. 7, A, B), though often 2 (Fig. 6, A, B) or 3 (Fig. 7, C) such filaments, which generally have no close mycelial connection with the oogonium, may contribute antheridial branches. Almost invariably the antheridial branches are not only wrapped about the oogonium itself, but are to some extent intricated with the filament supporting that organ; the intrication, however, being of an irregular or haphazard sort little suggestive of the symmetrical helicoid involvement characteristic of *P. helicoides* Drechsl. and *P. palingenes* Drechsl. Undersized oogonia may be supplied with only 1 (Fig. 6, C) or 2 (Fig. 7, A) antheridia, whereas larger specimens very often have 6 (Fig. 6, D), 7 (Fig. 6, B) or 8 (Fig. 7, B, C) male organs

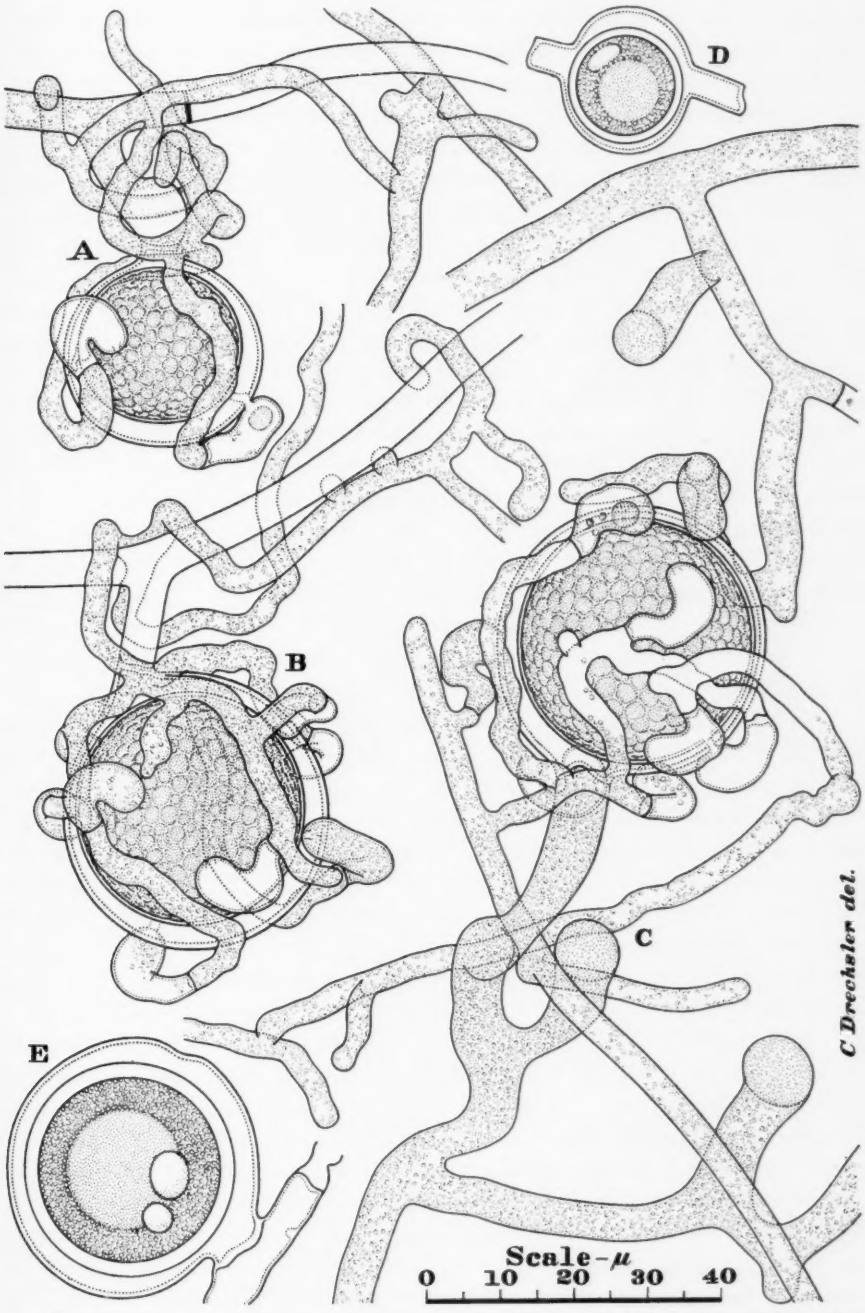

FIG. 7. Sexual reproductive apparatus of *Pythium myriotylum* formed in maize-meal-agar cultures; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A–C. Immature units showing antheridial relationships. D, E. Oogonia, each with a mature oospore.

formed in contact with them. Somewhat comparable arrangement of sex organs and associated hyphal parts is recognizable in *P. arrhenomanes*, *P. graminicolum* Subr., *P. periilum* Drechsl., and *P. scleroteichum* Drechsl., despite certain readily noticeable differences pointed out earlier (17, p. 885); and it seems probable that *P. myriotylum* is more closely allied to these species than to *P. butleri*, even though the latter offers much greater similarities of parasitic habit. Analogous enwrapment of the oogonium by its male complement appears suggested also in Matthews' illustrations (26, Pl. 11, Figs, 1, 2, 3, 6) of her *P. catenulatum*.

Like *Pythium arrhenomanes* and *P. scleroteichum*, though perhaps in lesser degree, *P. myriotylum* is annoyingly given to degeneration of its sexual apparatus. Often its oogonia form oospores that degenerate before the correct unitary organization of early maturity has been achieved. Indeed, frequently degeneration sets in before even an oosphere becomes recognizable. When this happens the affected oogonium may utilize, without much evident advantage, some or all of its contents in putting forth a disorderly array of hyphal outgrowths; or, again, it may suffer spectacular invasion by a tangle of hyphae often originating from one or several of the attendant antheridia. Occasionally, the tangled mass of hyphae gives rise to a new oogonium and new antheridia, which then proceed with normal development; so that eventually an oospore of small size, yet of correct internal structure, comes into being within the old oogonial envelope.

When cultures of *Pythium myriotylum* show much degeneration they usually show also a pronounced tendency toward morphological extravagances that finds some expression even in those units of sexual apparatus that yield oospores of correct internal structure. Many of the antheridia may then be abnormally long and conspicuously upcurved or contorted, while an excessively large proportion of the oogonia and oospores may depart widely from average dimensions. Relatively large dimensions of both oogonia and oospores often accompany degeneration caused by low temperature and nutritional deficiency. Thus, when grown at 14° C. on filtered maize-meal-decoction agar the strain of *P. myriotylum* isolated from a decaying cucumber in 1924 gave 28.8 μ as the average diameter of good oospores, and 35.4 μ as the average diameter of mature oogonia. When the same strain was grown at 28° C. on maize-meal agar that contained in suspension a substantial quantity of finely divided maize-meal, and that had been slightly acidulated with hydrochloric acid, measurements on 200 sexual units selected at random gave values of 20.8 μ and 26.5 μ for average diameter of oospore and oogonium, respectively. The smaller values were mentioned in the diagnosis of the species, as they were derived from material evidently far more representative of normal development than the material that had given the larger values. For the cultures grown at the higher temperature on the more nutritious medium yielded sexual apparatus in extraordinary abundance and with virtually no degeneration; whereas, those grown at the lower temperature on the filtered medium yielded such appa-

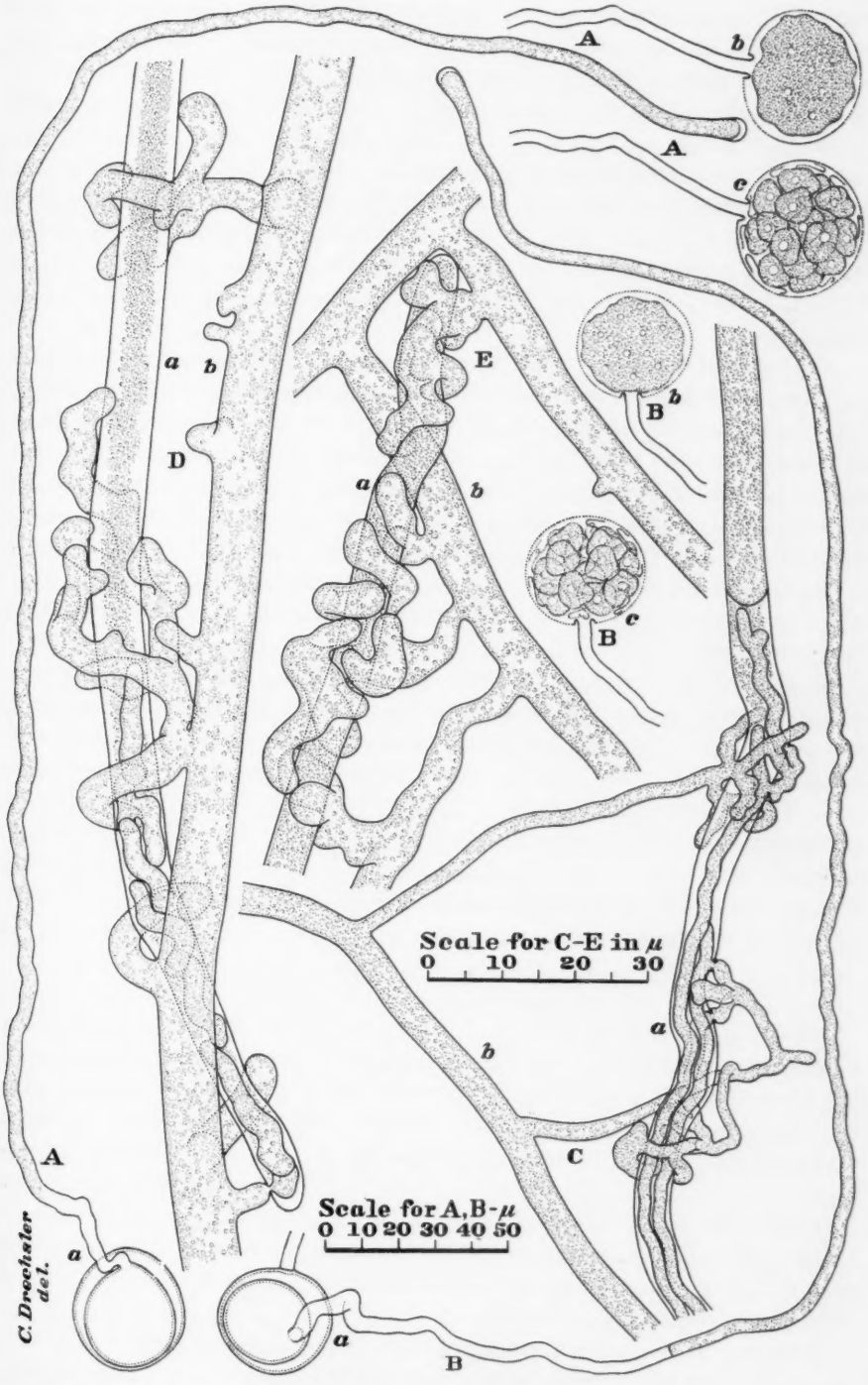

FIG. 8. A, B. Oospores of *Pythium myriotylum*, each germinating by production of a zoosporangium: a, long germ zoosporangium shortly before discharge; b, vesicle with discharged granular contents; c, vesicle with zoospores ready to escape; ×500. C. Hypha of *P. myriotylum* (a) parasitized by *P. periplocum* (b); ×1000. D. Hypha of *P. myriotylum* (a) attacked by pansy strain of *Aphanomyces cladogamus* (b); ×1000. E. Hypha of *P. myriotylum* (a) attacked by *Plectospira myriandra* (b); ×1000.

ratus only in moderate quantity, much of it vitiated by degeneration. Associated with the more abundant production of oogonia and oospores, and with virtual absence of degeneration, was a high degree of uniformity with respect to size. The 200 measurements of oogonial diameter from which the average of 26.5 μ was computed showed a distribution of values, expressed to the nearest integral number of microns, as follows: 16 μ, 2; 20 μ, 1; 22 μ, 1; 23 μ, 6; 24 μ, 7; 25 μ, 25; 26 μ, 54; 27 μ, 50; 28 μ, 29; 29 μ, 16; 30 μ, 5; 31 μ, 1; 32 μ, 1; 33 μ, 2; while the accompanying measurements for diameter of oospore showed the following distribution: 12 μ, 1; 15 μ, 2; 17 μ, 1; 18 μ, 6; 19 μ, 21; 20 μ, 41; 21 μ, 67; 22 μ, 37; 23 μ, 15; 24 μ, 6; 25 μ, 2; 26 μ, 1.

Oospores of correct internal structure have been found capable of germination after 2 or 3 years of storage at temperatures between 5 and 10° C. Like the reproductive bodies, sexual or asexual, of oomycetes generally, they produce vegetative germ hyphae when transferred to a fresh substratum devoid of free liquid water, or to a liquid medium containing a considerable quantity of nutrients in solution. When transferred to water containing little or no food substance they germinate more frequently by putting forth a simple filament, which in several observed instances was 500 to 600 μ long and 3 to 4 μ wide. After the protoplasmic contents have completely passed from the chamber of the oospore, and sometimes have further been evacuated from a proximal portion of the filament, the tip of the filament forms a hyaline cap of dehiscence (Fig. 8, A, a; B, a). Discharge of the germ sporangium thus brought into being proceeds as with zoosporangia of mycelial origin; the hyaline cap becoming inflated into a vesicle as it receives the migrating granular material (Fig. 8, A, b; B, b). The undifferentiated protoplasmic mass undergoes division into a swarm of laterally biciliate zoospores (Fig. 8, A, c; B, c), numbering usually 10 to 16 individuals, which escape when the vesicular membrane finally yields under the impact of their increasingly violent movements.

A NEW PROLIFEROUS PYTHIUM OF THE HELICOIDES SERIES

In June, 1939, A. A. Dunlap of Texas Agricultural Experiment Station submitted for identification a culture of a pythiaceous fungus that, according to an accompanying letter, had been isolated from roots of wheat plants sent from the Panhandle region of Texas. Since the numerous oospores visible in the substratum were unmistakably of multiplicate internal structure the fungus was at once presumed to belong with the series of proliferous forms typified in *Pythium helicoides*. This presumption was found amply justified when the asexual reproductive stage came to light. As a member of the *helicoides* series the fungus invites attention by unusual simplicity in the make-up of its sexual apparatus.

Growing at moderate temperatures in pure culture on agar media soft enough to offer no appreciable impediment, the fungus from Texas extends its mycelium more slowly than most species of *Pythium*. A relatively rich

medium, as for example, Lima-bean agar, induces development of rather copious aerial mycelium both in Petri dishes and in test tubes. Such aerial mycelium has been observed to persist more than 2 years under fairly dry conditions, without matting down on the substratum. Some aerial growth may often be observed also when the fungus is propagated in test tubes on maize-meal-decoction agar, a medium containing nutrients in moderate or in small concentration; though in Petri dishes this substratum usually affords development only of intramatrical mycelium that presents to the naked eye a somewhat lustrous radiating appearance modified occasionally by cloud-like variegation expressive of local differences in density of texture. Under the microscope, as might be expected, the intramatrical filaments are revealed, on the one hand, as being less haphazard in their courses than the filaments making up the diffuse mycelium of *P. myriotylum*, while, on the other hand, they show less parallelism of arrangement than is evident in the conspicuously lustrous mycelia of *P. vexans* de Bary (=*P. complectens* Braun) and *P. complens*. Intramatrical hyphal branches in plate cultures ordinarily fail to form appressoria on encountering the glass floor of the Petri dish,—a failure contrasting markedly with the behavior of the closely related *P. polytylum* Drechsl. (14), which, under similar conditions, gives rise to numerous adhesive organs.

Asexual reproduction may conveniently be induced by excising sizable slabs from a Lima-bean-agar plate culture permeated with young mycelium, and transferring them to a shallow layer of sterile water in a sterilized Petri dish. At temperatures between 20 and 25° C. each irrigated slab promptly extends into the surrounding liquid a fringe of extramatrical mycelium often 5 to 10 mm. in width. Within perhaps 15 or 18 hours after the transfer some of the filaments composing the fringe give rise individually to a terminal enlargement with a papillate protrusion at its apex (Fig. 9, A). Once it attains definitive size the enlargement is delimited from the supporting filament through deposition of a basal septum (Fig. 9, B, C, D). The terminal papilla may become prolonged into an evacuation tube of somewhat variable length. Should this tube suffer functional frustration, 1 (Fig. 9, E) or 2 (Fig. 9, F) similar tubes are put forth from other positions. Sooner or later the undifferentiated protoplasmic content of the sporangium passes into a vesicle formed through inflation of a hyaline cap at the tip of the successful evacuation tube (Fig. 9, G). In this vesicle the granular protoplasm is fashioned into laterally biciliate zoospores, wholly after the manner typical of the genus *Pythium* (Fig. 9, H). Often, especially when discharge is delayed while one or more longish evacuation tubes are being put forth, a branch may grow out laterally from the supporting hypha, just below the base of the sporangium (Fig. 9, G; H; I; J, a). At other times, when discharge has been accomplished quickly by means of a short apical evacuation tube, a second sporangium may develop within the emptied envelope (Fig. 9, K), eventually to give rise to zoospores in a vesicle formed a variable distance from the orifice of the first sporangium (Fig. 9, L).

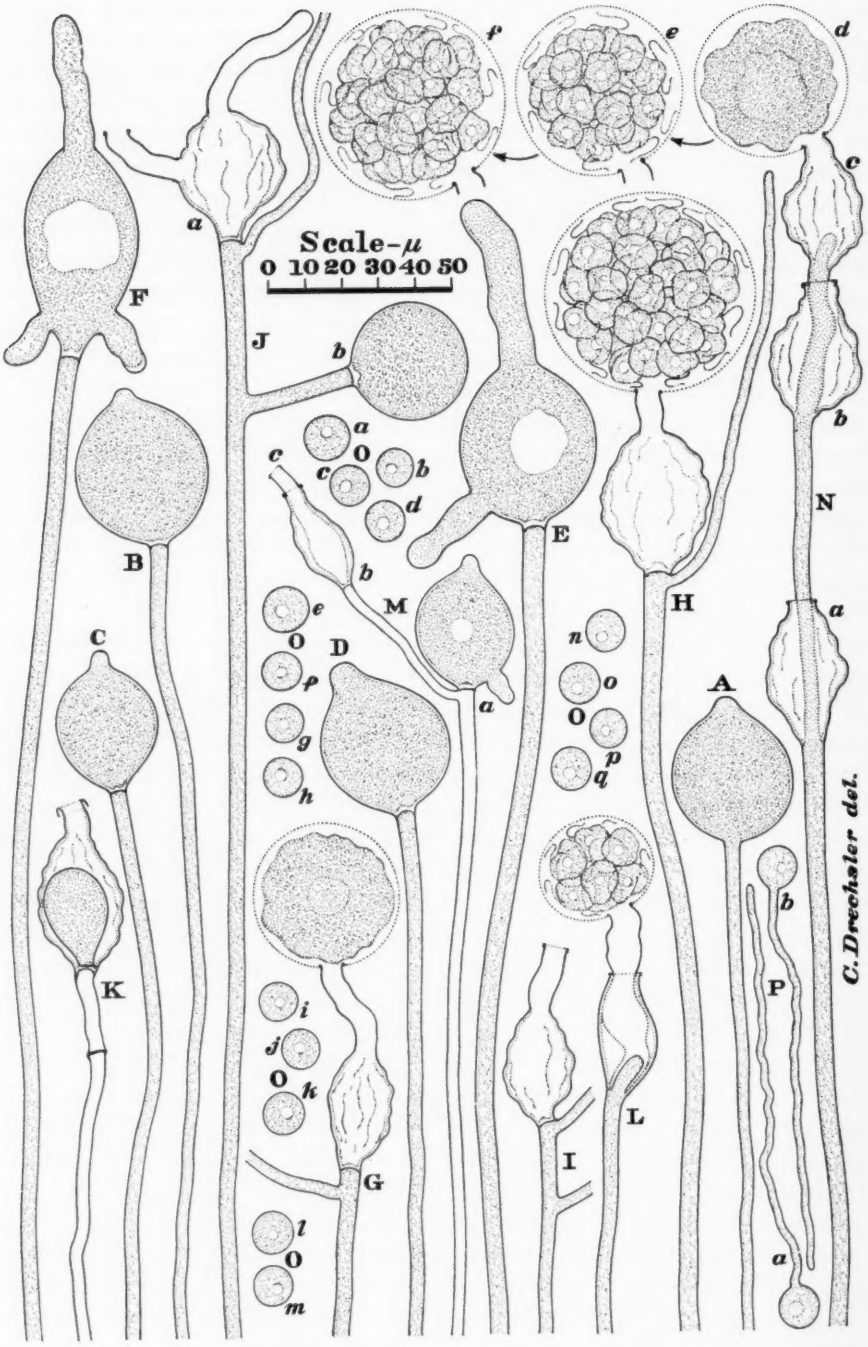

FIG. 9. Asexual reproductive apparatus of *Pythium ostracodes* produced in irrigated Lima-bean-agar preparations; drawn to a uniform magnification with the aid of a camera lucida; ×500 throughout. A. Young sporangium continuous with supporting hypha. B–D. Full-grown delimited sporangia. E, F. Sporangia with evacuation tubes. G–J. Discharged sporangia. K–M. Proliferous development of sporangia. N. Three uniaxial sporangia, (a–c); same vesicle at successive stages (d–f). O. Encysted zoospores (a–q). P. Germinating zoospores (a, b).

Owing to differences in promptness of discharge a terminal sporangium (Fig. 9, M, a) may sometimes be found still retaining its contents, even when a second and a third sporangium, formed subsequently on a lateral prolongation of the supporting filament, are present only as nested envelopes (Fig. 9, M, b, c). Such nested arrangement betokens, in the main, rather prompt discharge, as does also a seriate uniaxial arrangement of empty sporangial envelopes, brought about by repeated straightforward prolongation of the supporting filament and production of successive sporangia (Fig. 9, N, a, b, c), which, one after another, produce vesicles wherein their undifferentiated contents are transformed into zoospores (Fig. 9, N, d, e, f). After being liberated the zoospores swim about for some time before coming to rest and rounding up into subspherical cysts (Fig. 9, O, a–q) that germinate commonly by the production of a single germ hypha about 1.8 to 2 μ in width (Fig. 9, P, a, b).

Thus with respect to the morphology and development of its asexual reproductive phase, the fungus appears closely similar to the 4 proliferous species I presented earlier (14) as members of the *helicoides* series. It perhaps resembles *Pythium oedochilum* Drechsl. and *P. palingenes* more closely than *P. helicoides*, since its sporangiferous filaments, like those of the former 2 species, are little given to racemose or cymoid branching, even though now and then they may bear an auxiliary sporangium on a short branch arising some distance below the terminal sporangium (Fig. 9, J, b).

The fungus seems unusually reliable in producing normal sexual apparatus (Fig. 10, A–I; Fig. 11, A–G) on different kinds of agar culture media. On maize-meal agar containing in suspension a substantial quantity of finely divided maize-meal, its sexual development is, as a rule, not only very abundant but also to an extraordinary degree free of degeneration. The oogonium often arises as a lateral subspherical enlargement sessile on the parent filament (Fig. 10, A, B, C; Fig. 11, C); or it may be formed terminally either on a short branch (Fig. 10, D; Fig. 11, A, B), or on a longer hypha (Fig. 10, G); or, again, it may develop as a laterally intercalary (Fig. 10, E, F, I; Fig. 11, E, F) or mesially intercalary (Fig. 10, H; Fig. 11, D) body. During its earlier stages of growth it seems usually to have no direct contact with any part that can be distinguished as a male element (Fig. 10, A). On attaining definitive size it is fertilized commonly by a single antheridium (Fig. 10, B–I; Fig. 11, A–F), and only occasionally by 2 antheridia (Fig. 11, G, a, b).

Alongside of the robust oogonium the antheridium looks puny and frail, although actually it is not smaller than the corresponding organs in many of the more familiar species of *Pythium*. It consists usually of a somewhat swollen elongated cell formed terminally on a branch arising most often from the mycelial filament that either bears the oogonium directly (Fig. 10, B, C, E–I; Fig. 11, C, D, E), or gives off a short branch supporting the oogonium (Fig. 10, D; Fig. 11, A, B, G, a). In some units of monoclinous sexual apparatus, where the male branch is short and arises from a position

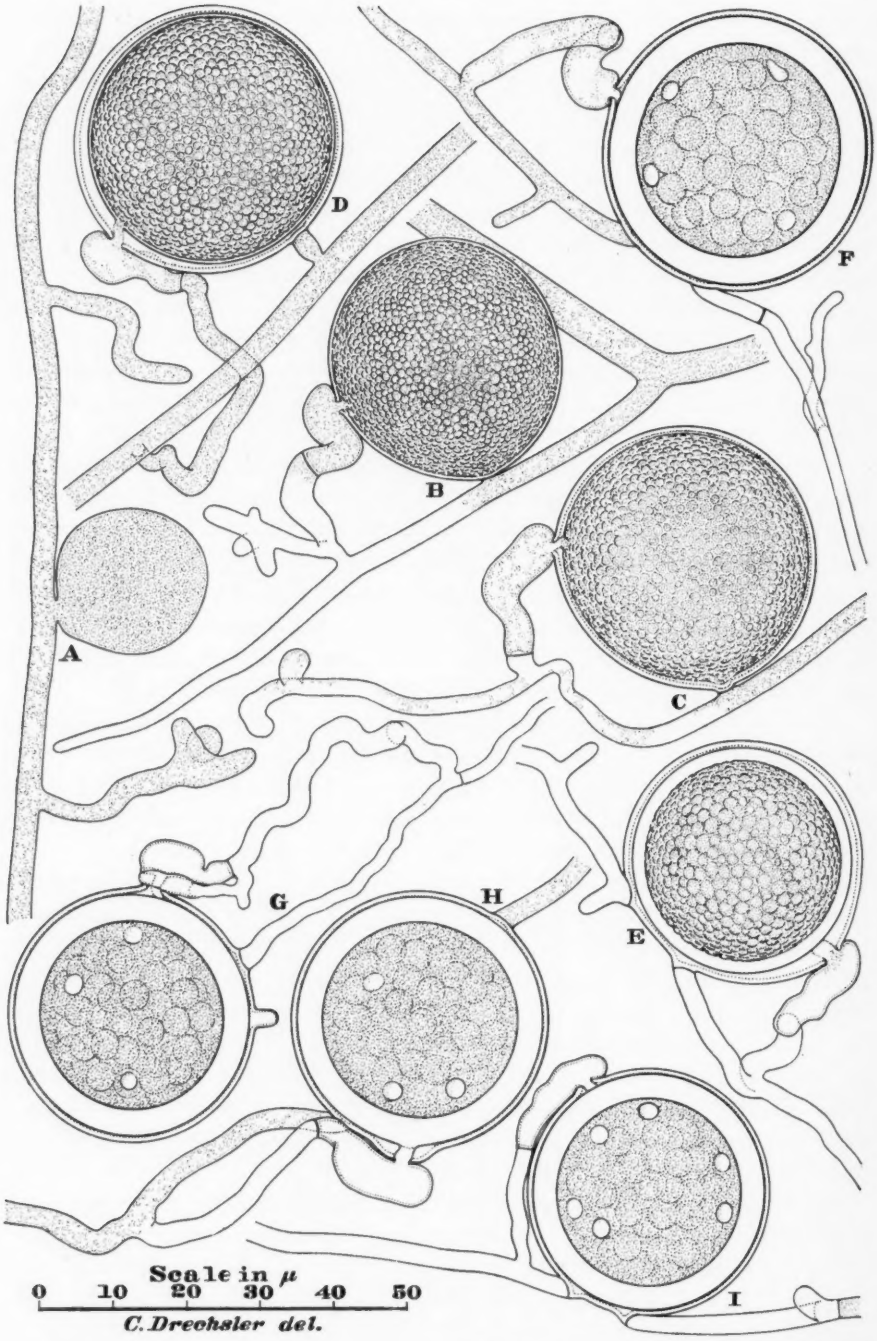

FIG. 10. Sexual reproductive apparatus of *Pythium ostracodes* formed in unfiltered maize-meal-agar medium; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Hypha with a young oogonium growing out laterally. B–E. Complete sexual units in immature condition. F–I. Complete sexual units, each with its mature oospore showing multiplicate internal organization.

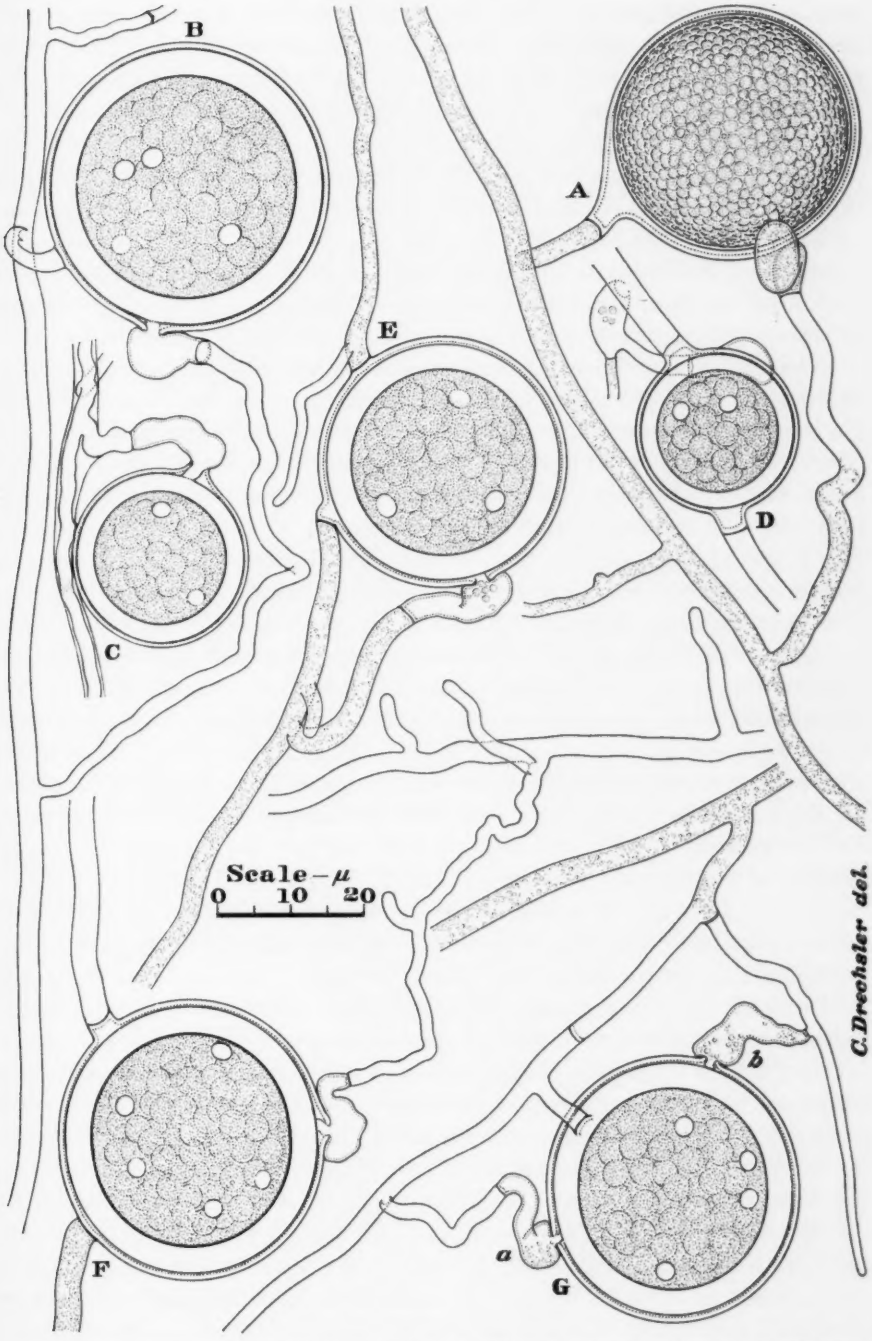

FIG. 11. Sexual reproductive apparatus of *Pythium ostracodes* formed in unfiltered maize-meal-agar medium; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Complete sexual unit in immature condition. B–G. Complete sexual units, each with its mature oospore showing multiplicate internal organization; the parent hypha in C illustrating the "Durchwachsung" often found in aging material.

close to the oogonium, the mycelial parts connecting the male and female organs may have an aggregate length of only 20 μ (Fig. 10, I), while in other monoclinous units, where the male branch arises from a more distant position, the mycelial connection between oogonium and antheridium may exceed 125 μ (Fig. 11, A) or even 150 μ (Fig. 11, B) in length. In units of diclinous sexual apparatus the length of the antheridial branch is rarely less than 50 μ (Fig. 11, F), and sometimes exceeds 200 μ. Now and then an antheridium may be found attached laterally (Fig. 11, G, b),—a positional relationship that might come about either from lateral development in the beginning, or from terminal development followed by renewed elongation of the supporting branch.

The antheridium is often found applied to the oogonium laterally almost throughout its length (Fig. 10, D, H, I), much as in *Pythium helicoides*. No less often, however, it is applied laterally only along its distal portion (Fig. 10, B, E; Fig. 11, B, F); and frequently, indeed, it merely makes apical contact (Fig. 10, C, F; Fig. 11, G, a, b) in somewhat the same commonplace manner as the antheridia, for example, of *P. dissotocum* Drechsl. (21, Fig. 2, 3). Although in some instances the oogonium comes to protrude rather markedly toward the antheridium (Fig. 10, E, H; Fig. 11, C) and thus provides obvious parallelism with *P. helicoides* and *P. oedochilum*, more often the wall of the globose body bulges out only slightly where the short fertilization tube is thrust through it (Fig. 10, F, I; Fig. 11, E, G, a, b). At the time of fertilization the oogonial contents appear to consist largely of protoplasmic blocks or lumps (Fig. 10, B, C). The lumpy mass often shrinks away perceptibly from the oogonial membrane (Fig. 10, D; Fig. 11, A) before its envelopment in a thick peripheral wall of its own (Fig. 10, E) heralds the internal changes required for its conversion into an oospore of multiplicate structure (Fig. 10, F–I; Fig. 11, B–G).

Usually only 2 to 6 refringent bodies are discernible in the oospore at early maturity. This number, however, includes only the refringent bodies lying close to the spore surface nearest the observer, since those in deeper positions are at this stage rather effectively obscured by the overlying granular material and reserve globules. The reserve globules, while noticeably larger than the refringent bodies, appear smaller and correspondingly more numerous than the multiple globules in *Pythium helicoides*. As a rule they have a diameter approximately equal to the thickness of the oospore wall, which dimension is even greater here than in the 4 closely related forms previously described. The oospore usually fills the oogonial chamber so nearly completely that its distinctly yellowish wall very often appears extensively in contact with the colorless oogonial membrane; the structural separateness of the 2 envelopes then remaining recognizable chiefly because of their difference in coloration. With respect to its main dimensions the sexual apparatus ordinarily is given only to moderate variation. The relevant metric data submitted in the diagnosis below were derived from 200 measurements of oogonia selected at random in a culture showing abundant

sexual development with virtually no degeneration. These 200 oogonia gave values for diameter, expressed to the nearest integral number of microns, distributable as follows: 14 μ, 1; 18 μ, 1; 21 μ, 1; 24 μ, 1; 25 μ, 1; 26 μ, 1; 29 μ, 1; 30 μ, 1; 31 μ, 2; 32 μ, 5; 33 μ, 22; 34 μ, 21; 35 μ, 32; 36 μ, 40; 37 μ, 27; 38 μ, 17; 39 μ, 10; 40 μ, 10; 41 μ, 2; 42 μ, 2; 43 μ, 2; and the 200 oospores of correct internal structure contained within them gave measurements for diameter distributable thus: 13 μ, 1; 16 μ, 1; 19 μ, 1; 22 μ, 1; 23 μ, 1; 26 μ, 1; 27 μ, 1; 28 μ, 1; 29 μ, 3; 30 μ, 3; 31 μ, 16; 32 μ, 31; 33 μ, 25; 34 μ, 38; 35 μ, 26; 36 μ, 22; 37 μ, 11; 38 μ, 10; 39 μ, 4; 40 μ, 1; 41 μ, 2.

Maize-meal-agar cultures containing numerous oospores of correct internal organization have, on examination after 2 years of storage at temperatures between 5 and 10° C., shown relatively little degeneration among the reproductive bodies. The conspicuously thick-walled structures seem, indeed, well designed for conserving life over long periods of time. Yet despite their longevity and their strongly indurated appearance the oospores will not only germinate very promptly, but, what is perhaps more remarkable, will germinate by producing zoosporangia and zoospores in almost all instances where liquid water moderately free of nutrients is present. Thus, when the oospores produced in 0.03 cc. of unfiltered maize-meal-agar medium are distributed in 3 cc. of water over the floor of a Petri dish 90 mm. wide, motile zoospores begin to be liberated after 6 or 7 hours, and in 15 hours may usually be found swarming in spectacular abundance throughout the preparation. Such behavior suggests that under natural conditions the fungus may possibly give rise to zoospores on a greater scale in connection with the germination of its oospores than through development of sporangia from its mycelium.

The first change unmistakably associated with germination becomes evident when the oospore wall—or, rather, the thick shell-like layer over the thin membrane intimately surrounding the protoplast—shows within a region between 5 μ and 10 μ wide an array of striations extending radially from its inner contour half way toward its outer contour (Fig. 12, A). These striations signify apparently that the wall is being progressively honeycombed with radial pockets. As the striations elongate and reach the outer contour, the remnants of wall material along the inner contour vanish, leaving a space into which the protoplast and the thin pliable membrane surrounding it soon protrude (Fig. 12, B). Solution of the honey-combed fabric continues centrifugally, and soon a fairly wide channel is cleared, permitting protrusion of the protoplast to the oogonial membrane (Fig. 12, C). Meanwhile, the portion of this membrane lying in the path of the channel has become somewhat less distinct optically, and now gives an impression of reduced firmness. At all events, it yields without much resistance in allowing the protrusion to break through and to elongate externally as a stout germ tube (Fig. 12, D). Thereupon radial striations come into view everywhere along the inner contour of the thick oospore wall (Fig.

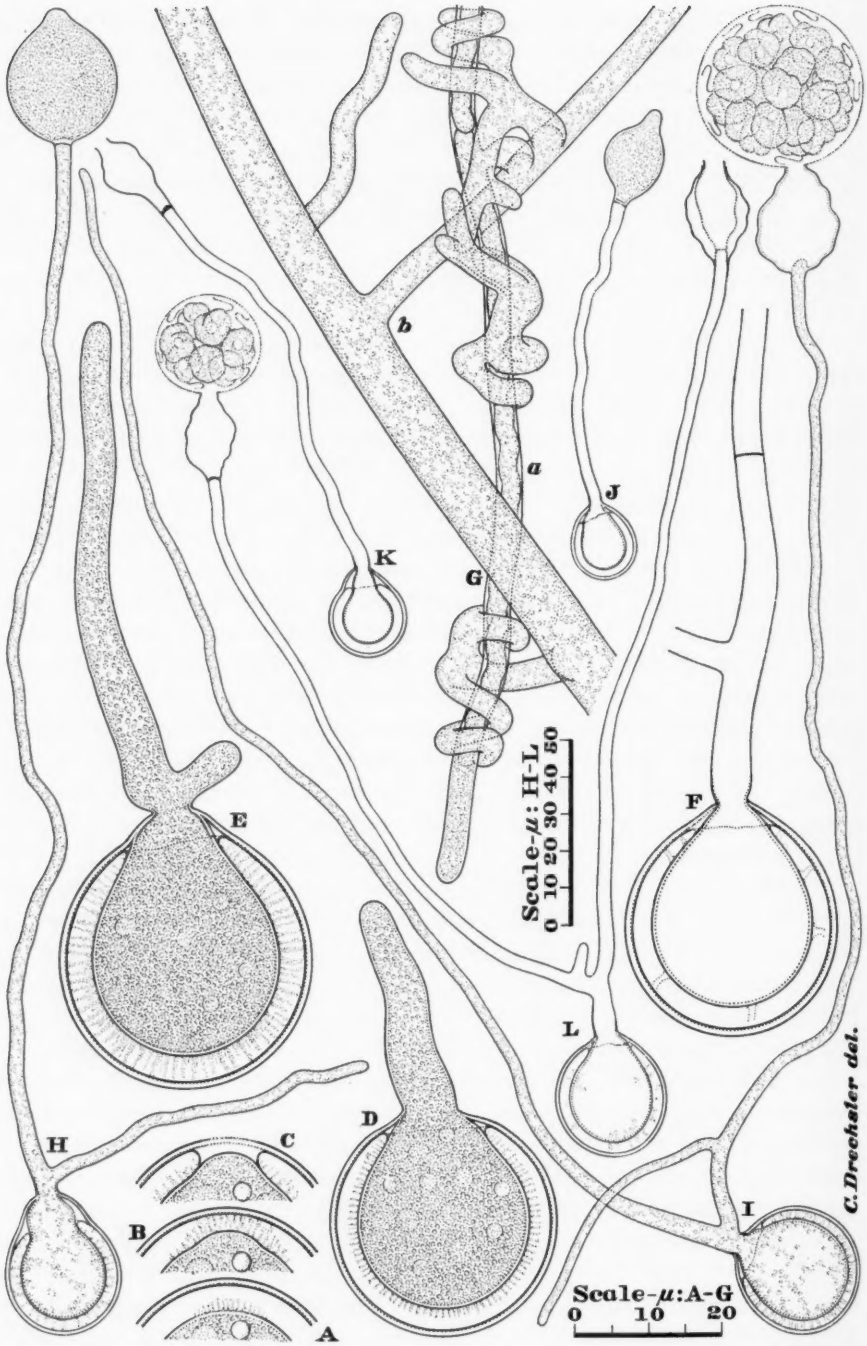

FIG. 12. *Pythium ostracodes.* A–C. Successive stages in perforation of oospore wall preparatory to germination; ×1000. D, E. Oospores with elongating germ tubes; ×1000. F. Membranous remains of oospore after germination; ×1000. G. Hypha of *P. ostracodes* (a) parasitized by spinach strain of *Aphanomyces cladogamus* (b); ×1000. H, I. Oospores which after forming one sporangium are ready to form other sporangia; ×500. J, K, L. Oospores which have exhausted their contents in forming 1, 1, and 3 zoosporangia, respectively; ×500.

12, D), and are soon extended to its outer contour (Fig. 12, E). Thus honeycombed throughout, the substance of the massive wall undergoes resorption, until finally only some few radial markings may remain as vestiges in the space between the persistent oogonial membrane and the similarly persistent membrane which intimately surrounded the protoplast (Fig. 12, F); and very often, again, even such meager vestiges are extinguished.

At about the time the first radial markings can be observed in a localized region of the massive wall surrounding them, the protoplasmic contents of the oospore begin to show significant changes. The reserve globules appear less distinct and are reduced in volume, while simultaneously the granular material is increased in corresponding measure. Temporarily the refringent bodies in the deeper portions of the protoplasmic mass become more clearly visible, so that their total number, usually between 10 and 15, and less often between 5 and 10, or between 15 and 20, can now be determined. During the period when the germ tube grows to a length of 50 to 75 μ, the refringent bodies gradually are lost to view in their densely granular matrix (Fig. 12, D, E). Usually, during this early period, too, a branch is given off by the germ tube close to its origin (Fig. 12, E). The 2 resulting elements commonly grow in rather widely divergent directions (Fig. 12, H), one or the other often putting forth an additional branch (Fig. 12, I). When a filament has attained a length of 75 to 500 μ, or sometimes even a length of 700 μ or 800 μ, it gives rise at its tip to a terminally papillate sporangium (Fig. 12, H) similar in shape to the sporangia produced from vegetative mycelium, and, like them, after development of an evacuation tube with an apex of dehiscence, discharging its contents into a vesicle for transformation into laterally biciliate zoospores (Fig. 12, I). The largest sporangia borne on germ hyphae yield between 20 and 25 zoospores, yet the production of one such organ ordinarily leaves unexpended much of the protoplasm in an oospore of good size (Fig. 12, H, I), even if some undersized oospores are completely exhausted in producing individually a short germ hypha together with a single small sporangium that may yield only between 5 and 10 zoospores (Fig. 12, J, K). Most oospores, consequently, give rise to 1 or 2 additional sporangia, in many instances with a display of proliferous development. Thus, the second sporangium often is produced on a prolongation of the filament that bore the first, while a third may be formed elsewhere at the tip of a branch (Fig. 12, L); or proliferous development may be absent, the 2 or 3 sporangia being produced on different filaments of the branching apparatus.

The more commonplace type of germination by production of mycelia ensues, of course, when oospores are transferred to agar media devoid of free water, as also when they are put into rich liquid media or into water containing substantial quantities of nutrients. In the earlier stages of such vegetative germination, digestion of the thick oospore wall proceeds much as in germination under aquatic conditions; but soon after the germ hyphae have emerged they begin to take up food substances, thereby initiat-

ing a new cycle of autonomous growth. Yet a supply of fresh water or fresh substratum is not always necessary for germination. Often, while undergoing microscopic examination, oospores have been found germinating actively within 2 hours after they had been removed, completely dormant, from maize-meal-agar cultures 6 or 8 weeks old, even though the stale ambient in which they were formed and which still surrounded them not only had received no addition of fresh water but was slowly losing water through evaporation.

As the fungus, apart from other diagnostic features, would seem distinguished from all known species of *Pythium* by its remarkably thick oospore wall, it is described as a new member of the genus under a specific name meaning "testaceous."

Pythium ostracodes sp. nov.

Mycelium hyalinum, ramosum, in hyphis 1.8–6.5 μ crassis consistens; zoosporangiis sphaeroideis vel ellipsoideis vel citriformibus, 25–55 μ longis, 16–38 μ crassis, fere terminalibus, pluribus deinceps ex apice unius hyphae repullulantis saepe gignentibus, protoplasma eorum per tubulum in vesiculam fundentibus; tubulo 2–45 μ longo, 4–9 μ crasso, plerumque ex apice sporangii rarius ex latere ejusdem orto; vesicula 5–35 zoosporas gignente; zoosporis primo reniformibus, a latere biciliatis, mox quietis, globosis, plerumque 10–12.5 μ diam. Oogonia terminalia vel intercalaria vel lateralia, globosa, 14–43 μ (saepius circa 35.4 μ) diam., pariete 0.5–1 μ crasso circumdata; antheridiis plerumque singulis rarius binis, vulgo androgynis rarius diclinis, clavatis vel cylindratis, fere 10–20 μ longis, 4–7 μ latis, modo a fronte modo aliquatenus vel omnino a latere ad oogonium appositis, membrana saepius aliquanto undulata tectis; oosporis flavidis, sphaeralibus, vulgo 13–41 μ (saepius circa 33.5 μ) diam., muro 1.5–4.3 μ (saepius circa 3.6 μ) crasso circumdatis, 10–75 pilulas oleosas plerumque 3–4 μ crassas et 5–20 corpuscula nitida 2–3 μ crassa continentibus, prompte germinantibus denique aut mycelium emittentibus aut hypham fertilem simplicem vel saepius aliquantulum ramosam cum 1–3 zoosporangiis proferentibus.

Habitat in radicibus Tritici aestivi in Texas.

Intramatrical mycelium in transparent agar media frequently of somewhat lustrous radiate appearance, capable of approximately 15 μ radial extension in 24 hours at 24° C., composed of hyphae mostly 1.8 to 6.5 μ wide, the more delicate ramifications usually developed only in moderate quantity. Aerial mycelium sometimes absent, but at other times moderately or even rather abundantly developed, and then under conditions not too humid persisting long without collapsing.

Sporangia under aquatic conditions formed terminally on long, simple, or sparingly branched extramatrical hyphae, mostly 2.5 to 4.5 μ wide; subspherical, prolate ellipsoidal, or lemon-shaped, measuring usually from 25 to 55 μ in length and from 16 to 38 μ in transverse diameter; discharging their contents into a vesicle through an evacuation tube often somewhat reflexed, 2 to 45 μ long and 4 to 9 μ wide, usually formed as a prolongation of an apical papilla, but sometimes arising from a lateral position, especially after frustration of an apical tube; proliferous in moderate measure, either through subsporangial branching, or through uniaxial elongation of the supporting filament. Zoospores formed in numbers ranging from 5 to 35, kidney-shaped, laterally biciliate, on rounding up forming cysts mostly 10 to 12.5 μ in diameter, which germinate usually by putting forth a germ tube about 2 μ in width.

Oogonia sometimes formed terminally on lateral branches less than 25 μ in length, but more often borne directly on long filaments in terminal, lateral, laterally intercalary, and mesially intercalary positions; in some instances protruding noticeably toward the antheridium, yet generally subspherical, measuring 14 to 43 μ, mostly 30 to 41 μ (average 35.4 μ) in diameter; provided with a wall 0.5 to 1 μ in thickness. Antheridia usually occurring singly in relation to an oogonium, but sometimes 2 in number; usually formed terminally or somewhat laterally on a branch 2 to 100 μ long arising from the same hypha as the oogonium at a distance 5 to 100 μ from the oogonium, but ocasionally of diclinous origin and then usually borne terminally on a branch 50 to 250 μ long arising from a distant filament; elongated cylindrical, clavate, or saccate in shape, often with somewhat wavy contours; measuring usually 10 to 20 μ in length and 4 to 7 μ in width; sometimes applied apically, but at other times applied lengthwise for some distance below the apex, if not for the entire distance from apex to base; producing a fertilization tube often 1

to 3 μ long and 1 to 1.5 μ wide. Oospore usually distinctly yellowish, subspherical, measuring 13 to 41 μ, mostly 28 to 39 μ (average 33.5 μ) in diameter; provided with a wall 1.5 to 4.3 μ, mostly 3.2 to 4 μ (average 3.6 μ) in thickness; containing 10 to 75 reserve globules mostly 3 to 4 μ in diameter, and 5 to 20 globose refringent bodies 2 to 3 μ in diameter; germinating readily by producing a mycelium, or, under aquatic conditions, by extending a simple or somewhat branched sporangiophore, 75 to 800 μ long, whereon 1 to 3 sporangia are borne terminally, often in part through proliferous development.

Occurring in rootlets of *Triticum aestivum* in Texas.

BIOTIC RELATIONSHIPS WITH SOME OTHER ROOT-ROTTING OOMYCETES

As might be expected, agar plate cultures that have been prepared for the isolation of root-rotting or damping-off fungi by placing pieces of affected plant material on sterile medium often afford development of mycelia belonging to 2 or 3 separate species of *Pythium*. When the several mycelia present in a culture are all referable to such familiar pathogenic species as, for example, *P. ultimum* Trow, *P. debaryanum*, *P. irregulare* Buism. and *P. mammillatum* Meurs, no hyphal relationship expressive of parasitism comes to light. When, however, a growing mycelium of one or another of these species has occasion to meet a growing mycelium of *P. oligandrum* Drechsl. (14), a species with echinulate oogonia, which occurs rather frequently in diseased underground parts of many phanerogamic plants, the former is attacked by the latter in a violently parasitic manner. Everywhere in the region of encounter delicate ramifications of the spiny form enwrap the filaments of the other elaborately, soon penetrating in different places and intruding hyphal processes that grow lengthwise through the filaments to assimilate the degenerating host materials. Here and there the internal hyphae give off branches that push their way out through the enveloping host membrane and, after some elongation, bring about the destruction of other host filaments nearby. During their earlier stages of development oogonia, as well as conidia or zoosporangia, may be invaded, though oospores appear immune from attack once they are surrounded by their thick wall. Frequently the attack of *P. oligandrum* on the congeneric forms commonly associated with damping-off is so devastating that in extensive tracts of substratum they can give rise to only a few reproductive bodies.

The same destructive parasitism came to light again and again when *Pythium oligandrum* was intentionally planted in dual culture with the familiar pathogenic forms found attacked in isolation cultures. Among more than a score of additional congeneric species that were likewise grown in dual culture with *P. oligandrum*, a few were parasitized with similar violence. Most of the others, including *P. myriotylum*, suffered less severe, though readily appreciable, injury. The remaining forms, of which *P. ostracodes* may serve as a convenient example, incurred little or no injury, even though in some instances their hyphae were intricately enveloped by branches of the spiny form. *P. complens* at times attacked *P. oligandrum* on a small scale.

As *Pythium acanthicum* and *P. periplocum* Drechsl. in their morphology show close kinship with *P. oligandrum*, these two species, better known

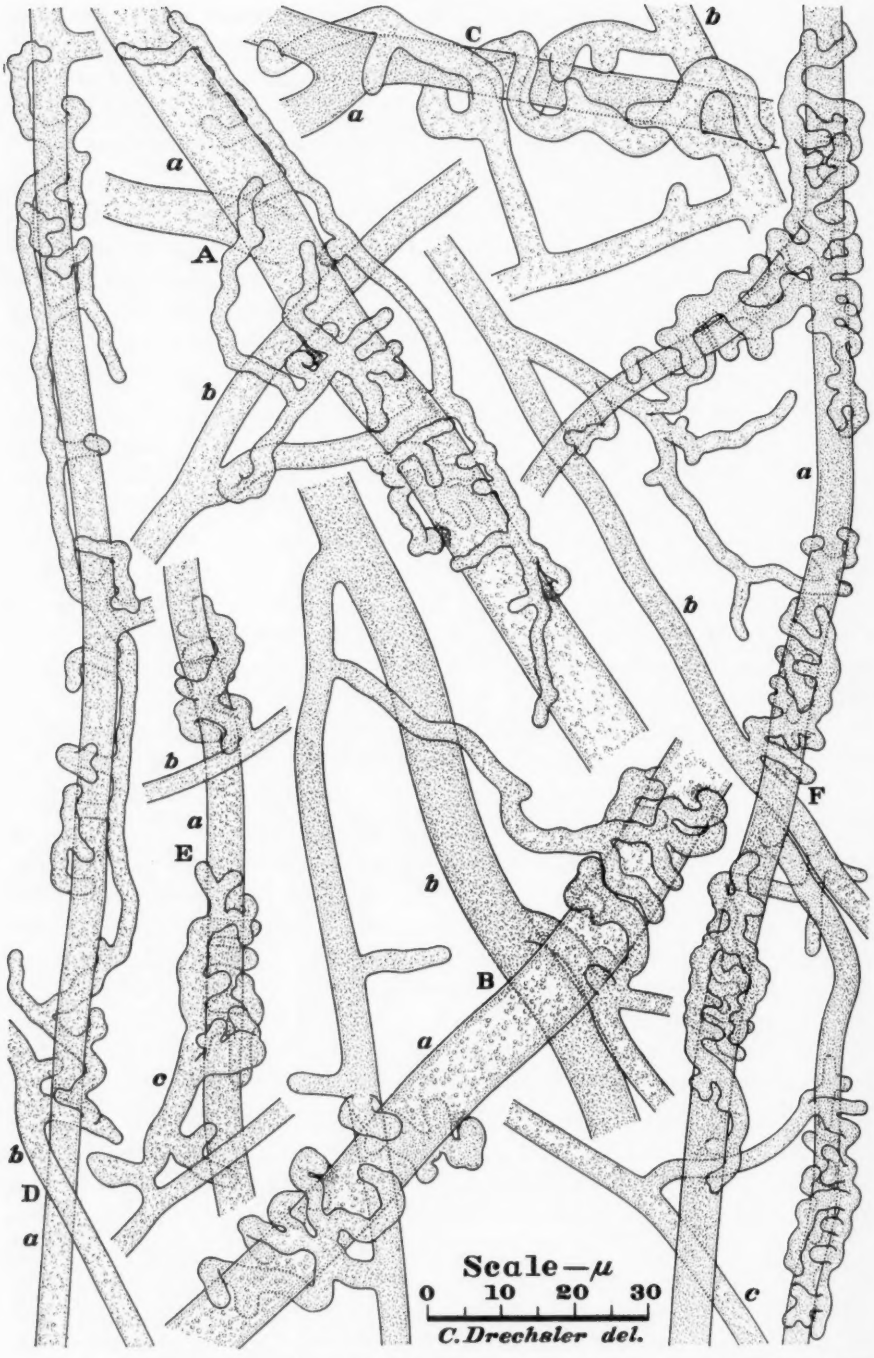

FIG. 13. Drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Hypha of *Pythium myriotylum* (a) attacked by *P. acanthicum* (b). B. Hypha of *P. myriotylum* (a) attacked by *P. oligandrum* (b). C. Hypha of *P. myriotylum* (a) attacked by spinach strain of *Aphanomyces cladogamus* (b). D. Hypha of *P. ostracodes* (a) scantily enwrapped by *P. periplocum* (b). E. Hypha of *P. ostracodes* (a) more densely enwrapped by *P. periplocum* (b, c). F. Hypha of *P. ostracodes* (a) enwrapped by *P. oligandrum* (b, c).

to me from their causal connection with blossom-end rot of watermelon than from their association with root decay, were also grown in series of dual cultures with a wide assortment of congeneric forms. In the 2 series of cultures parasitism and antagonism of varying degrees of severity again prevailed; the performance of the fruit-rotting fungi being generally similar to that of *P. oligandrum*. *Pythiogeton autossytum* Drechsl. (15), a member of the Pythiaceae, though alien to the genus *Pythium*, was attacked unmistakably. However, when the 3 echinulate forms were grown in dual culture with a number of saprolegniaceous forms occurring in association with root rot of crop plants, they not only proved innocuous, but like numerous other species of *Pythium* tried out, were themselves subjected to adverse action. In the case of *Aphanomyces cochlioides* Drechsl. (13) this action appeared to be mainly defensive, and caused little positive injury. Elaborate enwrapment of the pythiaceous filaments marked the more aggressive and injurious attack of *Plectospira myriandra* Drechsl. (11) as well as of the 3 strains of *Aphanomyces*, referable apparently to *A. cladogamus* Drechsl. (13) that were isolated, respectively, from diseased roots of pansies, *Viola tricolor* L. (16), spinach, *Spinacia oleracea* L. (18), and flax, *Linum usitatissimum* L. (18).

Pythium myriotylum, as has been intimated, is attacked by *P. acanthicum* (Fig. 13, A; Fig. 14, A), *P. periplocum* (Fig. 8, C; Fig. 14, B), and *P. oligandrum* (Fig. 13, B; Fig. 14, C, D) less severely than *P. ultimum* or *P. debaryanum*. On encountering its mycelium the spiny forms put forth numerous delicate branches that make contact with the filaments of *P. myriotylum*, and often enwrap them more or less extensively. Some of the delicate branches become slightly enlarged at the tip, which then is applied closely to the wall of the *myriotylum* filament, where often it is visibly cemented in place by means of an opaque secretion. The terminations thus present the appearance of appressoria, and, indeed, sometimes operate as such by intruding haustorial threads into the filament to which they are applied (Fig. 8, C; Fig. 14, D). These haustorial threads grow lengthwise within the invaded filament until their further extension in either direction is checked by a septum that the host has in the meantime laid down at some distance from the place of ingress. Since the granular contents of the host disappear locally as invasion proceeds, it may be presumed that they are assimilated by the haustorial threads, and that, accordingly, the relationship present here is an unambiguously parasitic one. Yet, in most cultures the greater proportion of *myriotylum* hyphae invested by branches of the spiny forms are not actually invaded. Often these hyphae show abnormal modification both in somewhat increased thickness of the peripheral wall generally and in much more pronounced local thickening on the inner side of the wall at places where appressoria have become attached. The protoplasm within them often remains in normal condition for a protracted period, but often, too, it migrates to other portions of the mycelium, or undergoes gradual degeneration. Similar migration and

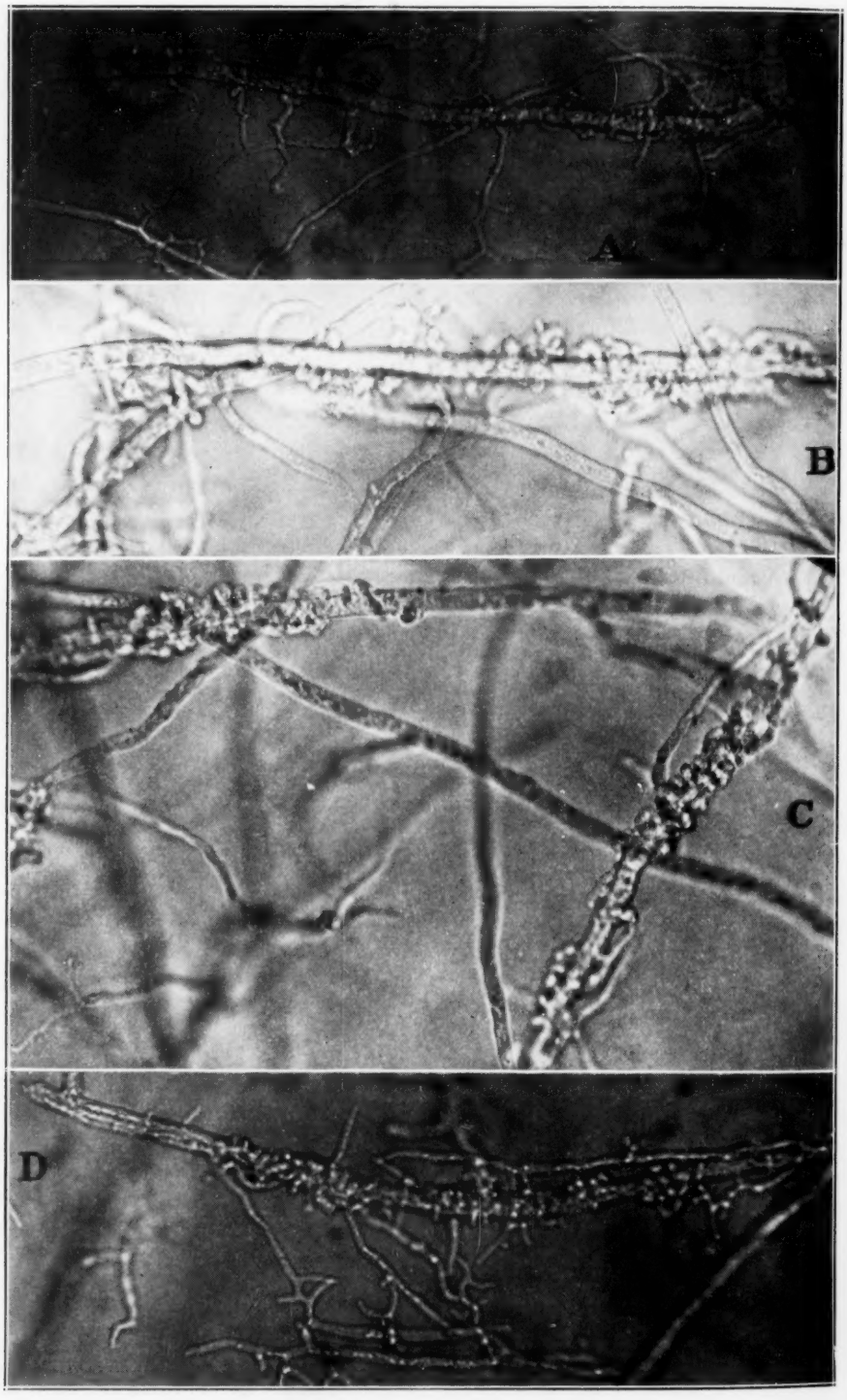

FIG. 14. A. Hypha of *Pythium myriotylum* attacked by *P. acanthicum*. B. Hyphae of *P. myriotylum* attacked by *P. periplocum*. C, D. Hyphae of *P. myriotylum* attacked by *P. oligandrum*.

degeneration of protoplasm takes place in some measure also in vigorous mycelium of *P. myriotylum* that, after being removed to a glass slide, is subjected to microscopical examination; so that the harmful effect of the enveloping branches is not easily ascertainable. Nevertheless the impression usually is gained that the branches are injurious, at least locally. It is not obvious, however, that the spiny forms obtain food materials from the *myriotylum* filaments merely intricated by them, unless, perchance, after the intricated filaments have been made to degenerate internally, their soluble contents may become available in part by diffusing out into the ambient. The expanded terminations that sometimes serve as appressoria seem hardly large enough to operate effectively as cupping organs.

When a growing mycelium of *Pythium myriotylum* encounters a growing mycelium of *Plectospira myriandra* (Fig. 8, E) or meets a growing mycelium of the pansy (Fig. 8, D), the spinach (Fig. 13, C), or the flax strain of *Aphanomyces cladogamus*, its hyphae in the zone of encounter are promptly enveloped in an elaborate manner by short ramifying branches, which, except for their greater width, resemble the branches extended under similar conditions by the 3 echinulate species of *Pythium*. As this envelopment proceeds the protoplasmic contents of the affected hyphae lose their normal appearance and become more or less opaque (Fig. 8, E; Fig. 13, C). Unmistakable disorganization promptly follows. Sometimes the enveloped portions of hyphae are, in addition, invaded internally (Fig. 8, D), but the injury accruing locally to the mycelium of *P. myriotylum* is obvious even where no invasion occurs. In any case the enveloped portions of hyphae, whether undergoing destruction through parasitism or through antagonism, are soon delimited from the adjacent healthy portions by cross walls.

Although *Pythium myriotylum* thus sustains some evident injury on encountering antagonistic oomycetous forms, its general development in dual culture is often impaired less than that of the fungus inflicting the injury. Under certain conditions of culture *P. acanthicum* and the strains of *Aphanomyces cladogamus* from pansies, spinach, and flax, are unable to advance any considerable distance beyond the zone of encounter into the tract of substratum already permeated with mycelium of *P. myriotylum;* whereas *P. myriotylum* will sooner or later spread over the tract of substratum originally occupied only by the opponent fungus. Under similar condition of culture, *Pythium periplocum* and *Pythium oligandrum*, as well as *Plectospira myriandra*, usually will halt the mycelial advance of *P. myriotylum* abruptly at the zone of encounter, while continuing their own advance into the tract already occupied by *P. myriotylum*. In such advance, however, their envelopment and destruction of hyphae diminish markedly, whether because they are adversely affected by the increasing staleness of the substratum, or because the older hyphae of *P. myriotylum* are less subject to attack. Meanwhile, as the older mycelium of the opponent species loses in vegetative vigor through aging and onset of sexual repro-

duction, the mycelium of *P. myriotylum* often recovers initiative and resumes growth by advancing in thinner array beyond the zone of encounter; so that, eventually, its oospores, like those of the opponent species, may often be found distributed in all portions of the culture. The ability of *P. myriotylum* to offset the discomfiture suffered in the zone of encounter seems attributable in part to its more prolonged vegetative period and its ready production of aerial mycelium.

When a growing mycelium of *Pythium ostracodes* (Fig. 15, A, a) encounters a growing mycelium of *P. acanthicum, P. periplocum* (Fig. 15, A, b) or *P. oligandrum* in a Petri-plate culture, both usually continue to advance without interference becoming manifest macroscopically in modification of their circular outlines. Indeed, on microscopical examination, sometimes only a few hyphae of *P. ostracodes* may be found enveloped by branches of the opposed spiny form. At other times, however, especially when a rather soft agar medium has been used in the preparation of the dual culture, numerous hyphae of *P. ostracodes* in the zone of encounter are very elaborately enveloped in ramifications of *P. periplocum* (Fig. 13, D, E), of *P. oligandrum* (Fig. 13, F; Fig. 16, A), or of *P. acanthicum.* On either side of the zone of encounter much less envelopment is noticeable; the intrication present in the tract where new hyphae from one of the 3 echinulate species have encroached upon aging hyphae of *P. ostracodes* usually being somewhat more abundant than that present in the tract where new hyphae of *P. ostracodes* have encroached upon old hyphae of the spiny form. It is remarkable that however elaborately the hyphae of *P. ostracodes* may be enveloped by any one of the 3 echinulate species, they commonly show no injury from such envelopment.

A far different result is obtained when *Pythium ostracodes* (Fig. 15, B, a) is grown in dual culture with *Plectospira myriandra* (Fig. 15, B, c; Fig. 16, B) or with any one of the strains of *Aphanomyces cladogamus* isolated from diseased roots of spinach (Fig. 12, G), flax, and pansies. In such dual culture the mycelial advance of *P. ostracodes* is abruptly halted wherever it encounters the mycelium of the saprolegniaceous form, which continues to grow with unabated rapidity. In the zone of encounter and soon afterwards also in tracts closer to their origin the hyphae of *P. ostracodes* are elaborately enveloped by branches of the opponent fungus, and their protoplasmic contents made to degenerate (Fig. 16, B). Here and there a filament may suffer internal invasion (Fig. 12, G). The injury thus sustained by the *Pythium* mycelium often results in noticeable reduction in the quantity of oospores formed by it.

Evidence indicating some sort of biotic relationship between oomycetes associated with damping-off and other diseases destructive to terrestrial phanerogamic plants was brought forward by de Bary (2) more than 60 years ago. In the garden cress (*Lepidium sativum* L.) seedlings that he used for substratum, this investigator never observed his *Pythium artotrogus* to occur alone: in all instances he found it accompanied by a congeneric

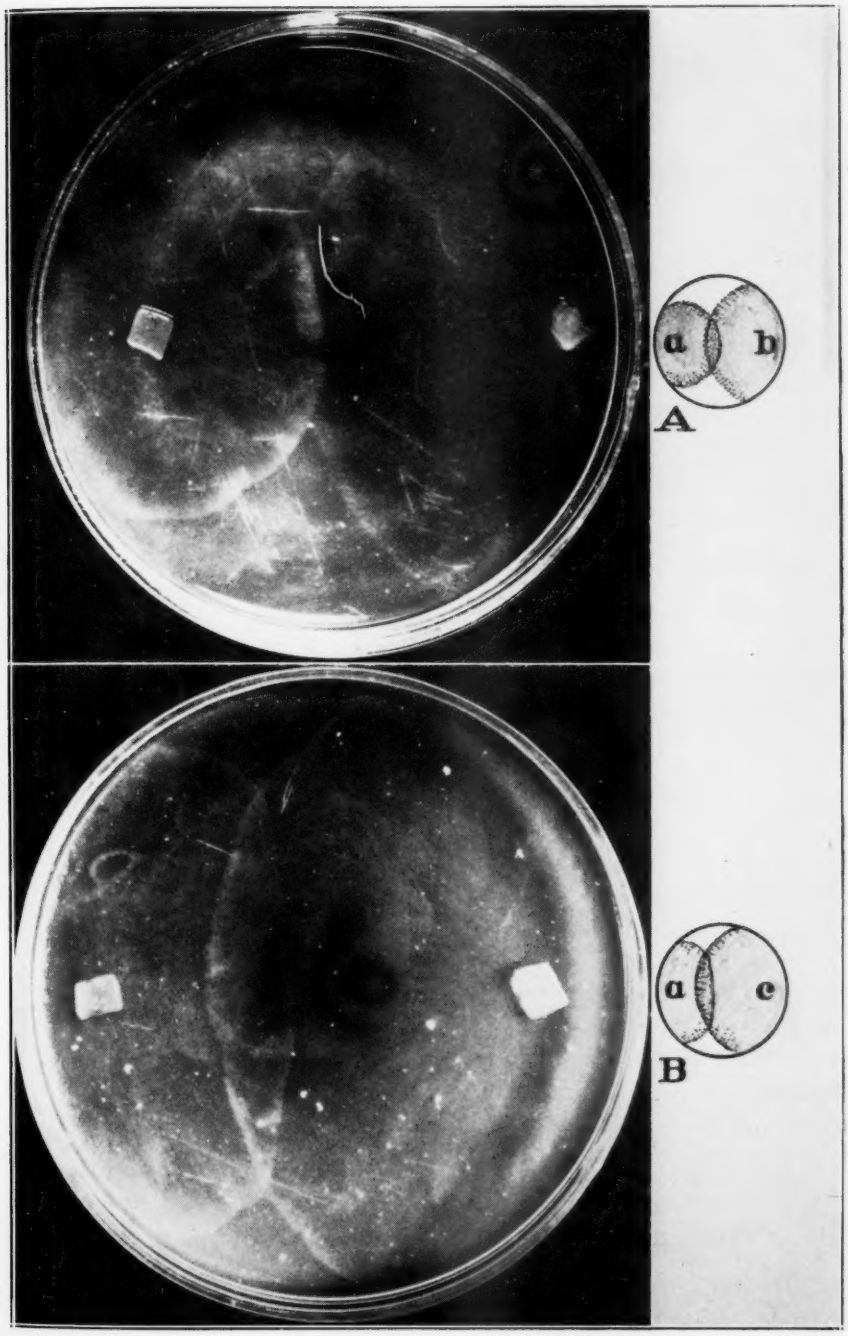

FIG. 15. Growing mycelium of *Pythium ostracodes* (a) encountering growing mycelium of *P. periplocum* (b), the rounded outlines of both mycelia showing no sign of retarded advance. B. Mycelium of *P. ostracodes* (a) encountering growing mycelium of *Plectospira myriandra* (c); the former, as is manifest from the lesser convexity of the portion of its outline within the region of encounter, being retarded or halted in its advance.

FIG. 16. A. Mycelium of *Pythium ostracodes* attacked by *P. oligandrum*. B. Hyphae of *P. ostracodes* attacked by *Plectospira myriandra*. The enveloped hyphae in A have remained normal, whereas those in B have collapsed.

species, which he identified as the widely pathogenic *P. debaryanum.* The congeneric species always appeared first, or at least became recognizable first through its earlier formation of reproductive organs. De Bary made repeated attempts to propagate *P. artotrogus* separately by removing living pieces of germ hyphae from its germinating oospores and placing them in drops of water to which were then added pieces of living and of scalded cress tissue. Though the germ hyphae branched abundantly in the liquid of the cultures thus prepared, the ramifying filaments consistently failed to infect either the living or the scalded tissue fragments, and consequently perished after a few days. Yet, in similar cultures to which *P. artotrogus* had been added, together with the related fungus recognized as *P. debaryanum,* the former always reproduced, even if at times only meagerly, by giving rise to its distinctive spiny oogonia.

De Bary offered 2 alternative explanations for the curious behavior of *Pythium artotrogus:* the fungus might require substances for growth that are absent in living or scalded cress tissue, but become available when such tissue is acted on by some other fungus, as, for example, *Pythium debaryanum;* or it might subsist as a parasite on *P. debaryanum,* much as species of *Chaetocladium* or *Piptocephalis* subsist as parasites on Mucoraceae. Further, de Bary had noted association of *P. artotrogus* with other fungi in its occurrence elsewhere than on cress seedlings in the laboratory. Thus, he had observed it associated with *Phytophthora infestans* (Mont.) de Bary in brown lesions on potato (*Solanum tuberosum* L.) stems, and with *P. infestans* as well as with *Pythium vexans* in dead tissue of potato vines. Again, in dried potato material in an authentic specimen of Montagne's *Artotrogus hydnosporus*—which species he held identical with his own *P. artotrogus*—he had observed spiny oogonia associated with swollen bodies of some alien fungus that might possibly have represented conidia of *P. debaryanum.*

De Bary's observations on *Pythium artotrogus* have been given some corroboration in several later writings. Butler's (4) report that he found *P. artotrogus* in rotting potato tubers attacked by *Phytophthora infestans* in Calcutta in 1902 tends to support the presumption of a habitual biotic association; though his further statement that for some time he successfully maintained the spiny fungus in cultures apparently free of *Phytophthora* hyphae, as also Mitra's (28) subsequent report that *P. artotrogus* had been found in stored potatoes in India, growing individually or associated with *P. infestans,* would seem at variance with the presumption of an obligate association. Under the binomial *Pythium hydnosporum* (Mont.) Schroet. supposedly the same spiny fungus was reported by Clinton (5) in Connecticut to have been observed not only in rotten potato tubers that had first been injured by *P. infestans,* but also in pea (*Pisum sativum* L.) roots injured by *Phytophthora cactorum* (Leb. and Cohn) Schroet., and especially abundantly in rotting grapes injured by the black-rot fungus and the grape berry moth. Sawada (31), working in Formosa, found *P. hydno-*

sporum in old tissue in a portion of a leaf of *Bochmeria nivea* Hook. and Arn. that had been attacked by *P. cactorum*. Dissmann (8), in Bohemia, while studying the relationship of premature decay of water lily (*Nymphaea candida* Presl.) foliage to the development, more particularly, of the 2 *Pythium* species he designated as *P. undulatum* Pet. and *P. proliferum* de Bary, noted that *P. artotrogus* sometimes occurred concomitantly with these 2 species, though only in older disintegrating portions of affected leaves,—a manner of occurrence he set forth as illustrating de Bary's view that the spiny form is adapted to the utilization of nutrient products supplied or modified by other fungi.

There is reason to suspect that among these additional first-hand reports on the occurrence of *Pythium artotrogus* in association with other fungi, as, indeed, also among the several first-hand reports mentioning its occurrence regardless of any such association, some may have been based on material not truly referable to de Bary's species. It is not evident that the different observers, with the exception of Butler (4), gave sufficient heed to the necessity for ascertaining whether their fungi showed correspondence with de Bary's (1) description in regard more particularly to fertilization of the oogonium by an antheridium consisting always of an adjacent hyphal segment, usually not modified externally, about 3 to 6 times longer than wide, and delimited by deposition of a septum in the supporting filament. Where determinations were based only on similarities to the oogonium of *P. artotrogus*—which organ was described originally (2) as being mostly 18 to 27 μ in diameter, and as being beset with tapering spines 3 to 6 μ long—the specimens might readily have belonged, instead, to *P. acanthicum*, *P. periplocum*, or *P. oligandrum*. In the morphology of their oogonia my 3 echinulate species show such close agreement with the illustrations and description of *P. artotrogus* that, from the beginning, I considered them intimately akin to de Bary's fungus. The agreement in morphology of oogonium would now seem to be sustained in parallelism of biotic relationship. This parallelism, it is true, would seem relieved by some noteworthy divergence. Contrary to de Bary's experience with *P. artotrogus*, my 3 species grow and reproduce well on many kinds of substrata, both natural and artificial, without the intervention of any other fungus; and 2 of them, moreover, are known to flourish under natural conditions as autonomous parasites on at least one phanerogamic host.

Attack on other phycomycetes by a saprolegniaceous fungus solely through external enwrapment of their hyphae was set forth by Couch (6) in the original descriptive account of his *Aphanomyces exoparasiticus*. Among the phycomycetes found subject to envelopment and injury was an unnamed species of *Pythium*. Although in one instance a direct protoplasmic connection was seen between a filament of this *Pythium* and a branch of *A. exoparasiticus*, no interchange of materials was observed. Apparently, *A. exoparasiticus* never invades the hyphae that it has enveloped, and that, as a result of being enveloped, have suffered disorganization

of their protoplasmic contents; so that, like *A. cochlioides* in some combinations, it would seem to lack even such scant internal assimilative apparatus as is formed under analogous conditions by *Plectospira myriandra* and by the 3 strains of *A. cladogamus* isolated from diseased roots of pansies, flax, and spinach. Unless the externally applied ramifications are capable of serving far better as cupping structures than appearances suggest, the elaborate attack of the several saprolegniaceous root-rotting forms here discussed would seem directed more to thwarting the development of competing fungi than to utilizing their substance as nourishment. In fine, the aggressive relationships displayed by these forms would seem to hold a larger measure of antagonism than of parasitism.

SUMMARY

Under warm, moist conditions, *Pythium myriotylum* produces abundant aerial mycelium, which, on encountering solid objects, gives rise to innumerable appressoria in dense clusters. This manner of growth makes possible the aerial parasitism whereby the fungus is enabled, much like *P. butleri,* to destroy bulked vegetables such as cucumbers, summer squash, and string beans. *P. myriotylum* rather closely resembles *P. butleri* also with respect to its asexual reproductive stage, though noticeable differences are present in the less pronounced inflation of its zoosporangial branches and in its somewhat less bountiful production of zoospores. Its sexual reproductive stage, however, differs greatly from that of *P. butleri,* seeming, rather, to betray intimate taxonomic kinship with *P. arrhenomanes.* The oospores, of unitary internal organization, often germinate, when bathed in fresh water, by putting forth a long simple germ hypha, which, after having received the entire protoplasmic contents, dehisces terminally as a zoosporangium.

A species of *Pythium,* isolated in Texas from wheat roots, is described as new under the binomial *P. ostracodes.* Its production of subspherical zoosporangia at the tips of hyphae, together with its development of oospores having multiplicate internal organization, characterizes it as a member of the *helicoides* series. In this series it is distinguished more especially by the very simple make-up of its sexual apparatus; fertilization being accomplished usually by a single antheridium borne terminally on a branch arising from the same hypha that supports the oogonium either directly or on a short stalk. When bathed in fresh water its oospores germinate very readily by producing, often in part through proliferous development, 1 to 3 terminal subspherical zoosporangia. During germination the very thick, shell-like, colored, outer layer of the oospore wall becomes conspicuously honeycombed with radial pockets and canals as its substance undergoes progressive resorption.

Pythium myriotylum, like numerous congeneric forms, is attacked by *P. acanthicum, P. periplocum,* and *P. oligandrum,* though less destructively than the familiar pathogenic species *P. ultimum, P. debaryanum,* and *P.*

irregulare. In their attack the 3 echinulate fungi send out numerous branches that invest the *myriotylum* hyphae, in some places merely bringing about noticeable abnormality, in some other places inducing local disorganization of protoplasmic contents, and in still other places intruding assimilative elements internally. The 3 echinulate species often envelop filaments of *P. ostracodes* more elaborately without, however, causing any appreciable injury. Further, the hyphae of *P. myriotylum* and *P. ostracodes*, as well as of many other species of *Pythium*, are subject to elaborate envelopment also by such saprolegniaceous root-rotting forms as *Plectospira myriandra* and the 3 strains of *Aphanomyces cladogamus* occurring in diseased roots of pansies, flax, and spinach, respectively. Invasion sometimes follows envelopment, but even when invasion does not occur the enveloped *Pythium* filaments suffer local disorganization of their contents. The seemingly meager utilization of the degenerating materials suggests that the relationship may be antagonistic more largely than parasitic.

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CORRECTIONS

In my paper entitled "Three species of *Pythium* with proliferous sporangia" (Phytopath. **31**: 478–507. 1941) the line preceding the last line on page 504, and reading, "sporangia, which often show proliferous development. In an old culture on" should properly follow the second line of text on page 505. A somewhat less bewildering error in line 20 on page 499 of the same paper may be corrected by changing the word "furthering" to "further."—C. D.

STUDIES ON GENOTYPES OF TOBACCO RESISTANT TO THE COMMON-MOSAIC VIRUS[1]

H. H. McKinney[2]

(Accepted for publication August 24, 1942)

INTRODUCTION

Resistance to common mosaic caused by Nicotiana virus 1 is manifested in several degrees in *Nicotiana tabacum* L. Clayton, Smith, and Foster (1) recognized three classes of resistance in terms of symptoms. Class 1 denotes none to very few markings; class 2, distinct diffuse spotting; and class 3, mild systemic mottling. A more detailed understanding of some of these classes, especially class 1, is desired from the standpoints of tobacco improvement, virus synthesis, and resistance. This report covers the results of a rather general survey to determine differences in the amounts of virus produced in some of the typical genotypes, and effects of age of plant on virus synthesis and movement. The studies were conducted at Arlington Farm, Virginia. A brief abstract of the results has been published (4).

FIELD STUDIES

Materials and Methods

Several resistant and two susceptible tobaccos were studied, but most of the work was done with (1) a selection of Ambalema; (2) T.I. No. 448, Sel. A; and (3) Wisconsin-Havana Seed. The selection of Ambalema used seems to be one of the most resistant yet isolated from the collections obtained by Nolla and Roque (8). This selection when infected usually manifests a few diffuse light-green spots on the leaves of young plants when grown in the field. In the greenhouse these spots occur less frequently. Mosaic mottling never occurs. T.I. 448 was collected in the vicinity of Mt. Tolima, Colombia, S. A. This area is not far from the area where Ambalema tobacco was collected in the Department of Valle del Cauca (8). Morphologically, the two collections used are nearly identical; they mature late and produce as many as 50 or more dark-green leaves. T.I. 448A has never exhibited mosaic mottling. Among more than 250 plants inoculated with the virus of common mosaic, only 12 manifested faint, light-green, diffuse zones on 1 to 3 leaves per plant. These were so inconspicuous that they would not be observed except with special care. These zones were not more frequent in the field than in the greenhouse. Wisconsin-Havana Seed tobacco is very susceptible to common mosaic, has a midseason-maturing habit, and produces 40 or more dark-green leaves.

[1] Studies supported in part by funds provided under Bankhead-Jones Project S.R.F. 2–17, U. S. Department of Agriculture, Bureau of Plant Industry and Bureau of Agricultural Chemistry and Engineering cooperating.

[2] Senior pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer wishes to acknowledge the assistance of Matthew Koerner in conducting the tests, and also the aid provided by E. E. Clayton of the Division of Tobacco Investigations in the matter of field facilities and plant material.

In 1937, a number of resistant collections were grown in the field. As soon as the plants were well established, after transplanting, they were inoculated with Nicotiana virus 1 by wiping the leaves. In late July and early August the plants were 6½ to 7½ feet tall. At that time, the three classes of resistance mentioned above were plainly evident. On August 3, ten plants from each of several collections within each class were individually assayed. The assay samples, two leaves (6 to 10 inches long) near the top of each plant were placed in tight screw-top bottles, brought to the laboratory immediately, and frozen. The procedures followed in obtaining the extracts and making the assays are described in the chapter on greenhouse studies.

In 1938, field-grown plants were studied as in 1937, except that the 10-plant samples were batched, and the upper and middle leaves were assayed separately. The plants had set many seed pods, and all leaves on the main stalk were fully developed.

Results

In tables 1 and 2 it will be noted that a total of 8 collections failed to manifest visible signs of infection. Three of these, T.I. 448A, T.I. 450, and

TABLE 1.—*Summary of assays for presence or absence of Nicotiana virus 1 in upper leaves of resistant classes of Nicotiana tabacum grown in the field in 1937, samples collected August 3. Ten plants of each plot were tested*

Variety or T.I. No.	Symptoms July 23 and Aug. 3	Lesions per bean plant from non-diluted juice of each tobacco plant	Tobacco plants with upper leaves free of virus
		Number	*Per cent*
448A	None	0	100
450	do	0	100
320	do	0 to 12	40
384A	do	0 to 27	20
Ambalema	do	0 to 100	10
439A	Severe diffuse spotting	100 to 550[a]	0
329	Mild mottling	100 to 550[a]	0

[a] Estimates based on sample counts.

T.I. 1110B, yielded no virus in the upper leaves late in the season, whereas Ambalema, T.I. 320, T.I. 384A, T.I. 470A, and T.I. 472A3, had virus in similar leaves in some of the plants.

In table 2 it will be observed that T.I. 448A was the only one that failed to yield virus from the leaf samples collected midway between the bases and tops of the plants late in the season. Of the resistant types, Ambalema carried the greatest amount of virus in these leaves.

The types manifesting severe diffuse spotting (T.I. 439A) and mild mottling (T.I. 329) in table 1, and the very susceptible variety, Maryland Medium broadleaf, in table 2 carried relatively large amounts of virus.

TABLE 2.—*Summary of assays for presence or absence of Nicotiana virus 1 in upper and in middle leaves of mature plants in resistant classes and in a susceptible variety of tobacco grown in the field in 1938*

Variety or T.I. No.	Symptoms Aug. 20 and 28	Lesions per bean plant from upper leaves of 10 tobacco plants. Collected Aug. 20. No dilution	Lesions per bean plant from middle leaves of 10 tobacco plants. Collected Aug. 28. Diluted 20 ×[a]
		Number	*Number*
448A	None	0.0	0.0
1110B	do	0.0	4.5
470A	do	0.6	3.9
472A3	do	2.0	0.1
Ambalema	do	3.0	140.0
Maryland Medium	Severe mottling	102.2	4.9[a]

[a] The extract from Maryland Medium was diluted 2×10^{-4} times in buffer. With a dilution of 20 × the lesions would be too numerous to count.

GREENHOUSE STUDIES

Materials and Methods

The plants were grown in earthen pots and transplanted to pots of increasing size, up to 8 inches in diameter, as their development required. Nicotiana virus 1 was used in all tests. Greenhouse temperatures were near 21° to 24° C., except when the solar energy was high. During the summer the glass was covered with a light sprayed mixture of cement, whiting, glue, and water. Temperatures were not controlled. Solutions of calcium nitrate were added to the soil at suitable intervals.

Leaf samples for assay were removed from well-watered plants that had been in the laboratory until turgid or when limp leaves were collected, they were placed with their cut ends in water in the laboratory and allowed to stand until turgid. This procedure tended to reduce some of the error in obtaining the tissue extracts. When small samples were studied, the fresh tissue was weighed and placed in a mortar with a small amount of phosphate buffer at pH 7.0. After some reduction, a little more buffer was added. Except when stated otherwise, this process was repeated until 3 parts of buffer had been added. The mortaring continued until the tissue had been reduced to very fine particles. The resulting soupy mass was poured into a double-cheesecloth-lined funnel and the liquid collected in a test tube. The extract was used for inoculations with or without further dilutions, depending on requirements. The assays were made on the primary leaves of bean plants held near 33.5° C. for 40 to 64 hours after inoculation (6).

Thorough grinding of the tissues is necessary to free the traces of virus frequently encountered in the resistant varieties. However, the method has been found to be the best yet devised for studying small samples of highly susceptible tissues as practically all virus is freed into the excess of liquid.

Of the several test plants studied, *Phaseolis vulgaris* L. (variety Scotia),

and *Nicotiana glutinosa* L., proved the most sensitive for detecting traces of virus, but in the case of the bean, satisfactory results depend to a large extent on the high culture temperature following inoculation.

When large samples were studied the fresh tissue was cut in very small pieces, placed in tightly stoppered bottles and kept frozen until tested. For testing, the tissue was pressed under hydraulic pressure and the extract was used, with or without further dilution, to inoculate the leaves of the test plants.

Experimental

Concentration of Virus in Leaves.—To determine the relative amount of virus recoverable from the top, center and lower leaves of T.I. 448A, Ambalema, and Wisconsin-Havana Seed, the young plants were inoculated April 1.

On May 30, leaves were removed from the bases, middle portions, and near the tops of 4 plants of each of the three types.

Plants of T.I. 448A and Ambalema ranged from 48 to 52 inches tall with no floral buds in evidence, and the plants of Wisconsin-Havana Seed ranged from 36 to 42 inches tall and were just beginning to bloom.

The topmost leaves sampled were 3 to 4 inches long; these, with 2 leaves directly below, made up the top-leaf sample. One middle leaf and a basal leaf were used from each plant. The basal leaves were just beginning to show the first senile yellowing and none of these had been wiped with virus. All midribs were discarded from the leaf tissue.

The corresponding samples from each of the 4 plants within each type were batched. All samples were finely cut with a sharp knife, placed in tight bottles, and frozen. On June 9, the samples were extracted, diluted in buffer, and inoculated on the primary leaves of Scotia bean plants. Owing to the large number of samples, only those dilutions were assayed that previous preliminary tests indicated would give the essential information on each plant type and leaf location. On June 15 another assay was made. The results are assembled in table 3.

These data indicate that the top leaves of T.I. 448A and Ambalema manifest less virus activity than the lower leaves, and that the virus content of T.I. 448A was less than that of Ambalema. As in the field material, the T.I. 448A plants in this test failed to yield detectable virus in the upper leaves.

In Wisconsin-Havana Seed, the first assay indicated the greatest amount of virus in the top leaves. However, the second assay indicated the greatest amount in the bottom leaves. Other tests indicate that the lower leaves of this variety yield the most active virus, but it appears that the difference is not always great.

Virus Increase in T.I. 448A.—To determine if virus can increase in the leaves of half-grown and of nearly mature healthy plants, and to obtain information on related phases, several experiments were conducted. In

TABLE 3.—*Results of assays for Nicotiana virus 1 in leaves from upper, middle and lower portions of stems of T.I. 448A, Ambalema and Wisconsin-Havana Seed tobacco. Tests conducted during spring and summer months, 1938*

Tobacco variety or T.I. number	Number of lesions per bean plant at indicated dilutions from—														
	Top leaves, diluted—					Middle leaves, diluted—					Basal leaves, diluted—				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
448A															
1st assay[a]	0.0	.....	.....	.....	.....	8.0	0.6	0.2	.....	.....	498	305	72	.....	.....
2nd do [b]	.....	.....	.....	.....	.....	0.9	.....	.....	.....	.....	.....	.....	.....	.....	0.2
Ambalema															
1st assay	4.0	.....	.....	.....	.....	355.0	221.0	67.0	.....	.....	.....	534	239	.....	.....
2nd do	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.3	.....	.....	.....	.....	1.8
Wisconsin-Havana Seed															
1st assay	779.0	825	467	.....	.....	.....	.....	400.0	114	.....	.....	.....	317	110	.....
2nd do	.....	.....	.....	.....	1.5	.....	.....	.....	.....	1	.....	.....	.....	.....	12.0

[a] Data in first assay were obtained on 5 plants (10 leaves) of Scotia bean plants for each diluted sample.

[b] Data in second assay were obtained on 35 half leaves of 70 Scotia bean plants. The samples were divided into two groups of 3 and 4 samples, respectively, and each sample within a group occurred on each of the 35 plants.

some cases comparisons were made with leaves of healthy Ambalema and Wisconsin-Havana Seed. Tests were made during the summer.

A nondiluted native extract of virus was wiped on 19 well-developed leaves of a single plant of T.I. 448A, and 12 to 19 days later each inoculated leaf was extracted, diluted to 3 volumes in buffered solution and wiped on 5 bean plants. Beginning with the lowest leaf position on the tobacco plant and proceeding upward, the 19 leaves induced lesions per bean plant, respectively, as follows: 22, 69, 84, 84, 33, 63, 29, 48, 14, 7, 11, 10, 59, 5, 9, 7, 23, 16, and 2. A few of the lesion counts cited are very low. It is possible that some of these lesions were from virus adhering to the tobacco leaves after wiping and do not represent virus increase. However, leaves were rinsed very thoroughly after wiping.

Very little detectable virus adheres to tobacco leaves when they are properly rinsed. Such leaves have been extracted immediately after being inocu-

TABLE 4.—*Relative amounts of virus detectable in the inoculated leaves of three types of tobacco when all leaves were inoculated as the plants were nearing maturity. Data in terms of lesions per bean plant. Extracts diluted 10^{-1} with buffer*

Leaf groups	Leaves in group	Lesions per bean plant from		
		T.I. 448A	Ambalema	Wisconsin-Havana Seed
	No.	*No.*	*No.*	*No.*
Upper	4	0.0	0.8	265.0[a]
Upper middle	6	0.6	0.5	283.0
Lower middle	6	4.7	11.1	381.0
Lower	6	16.5	6.0	136.0

[a] Because of the relatively low dilution, these counts are proportionally low in comparison with those from T.I. 448A and Ambalema.

lated and rinsed, and the extracts wiped on Scotia beans. Usually no lesions appear, but occasionally as many as 3 have been observed on a leaf.

In a second experiment, similar inoculations were made on the upper, virus-free leaves of nearly mature plants of T.I. 448A that had virus in the lower leaves. All the inoculated upper leaves gave evidence of supporting virus increase of the order shown in the above test on those plants that were virus-free throughout before the inoculation. Thus it seems evident that virus present in the lower leaves did not induce resistance in the upper leaves of T.I. 448A.

In a third experiment, T.I. 448A was compared with Ambalema and Wisconsin-Havana Seed. The plants were virus-free and were approaching the flowering stage. Beginning with upper leaves, which were 3 inches long, all leaves were wiped with a fresh native extract. Thirteen days later they were collected in groups of 4 to 6 leaves each and assayed on bean leaves. The number of lesions per bean plant induced by the extracts from the several leaf groups are assembled in table 4.

The fact that the extracts were diluted 10^{-1} in this test may explain the absence of detectable virus in the upper leaves of T.I. 448A. However, the

lower virus activity in leaves of the upper groups is evident, especially in the resistant types. The drop in activity in the lower leaves from Ambalema and Wisconsin-Havana Seed may be due to the fact that these leaves became senile earlier than the lower leaves of T.I. 448A.

Leaves 3 to 6 inches long at the top of vegetating tobacco plants are difficult to wet because of the excessive waxy exudate and the compactness of the trichomes. This may explain some of the reduction in lesion counts on beans inoculated from these leaves. These factors, however, would not involve more than the top 3 or 4 leaves wiped. The topmost group of leaves on the Wisconsin-Havana Seed produced much virus (Table 4).

Progress of Virus in the Plant.—To determine the progress of virus in T.I. 448A and Ambalema when inoculated in an advanced stage of development, a number of tests were made in comparison with Wisconsin-Havana

TABLE 5.—*Progress of Nicotiana virus 1 in large plants of T.I. 448A and Ambalema tobacco as evidenced by the number of lesions per bean plant induced by nondiluted leaf extract from selected tobacco leaves above and below the zones of inoculation. Assays were made 48 days after inoculation. Samples diluted in 3 parts of buffered solution*

Leaf positions[a]	Lesions per bean plant from virus from						
	T.I. 448A, plant No.				Ambalema, plant No.		
	I	II	III	IV	I	II	III
32	0	0	0	0	0	0	0
26	0	0	0	0	0	11	0
22[b]							
16	6	2	10	3	0	33	7
12	1	0	0	0	0	8	11

[a] Leaf positions are listed by leaf number, counting from the base of the plant, at time of assay.

[b] Inoculated leaves.

Seed. The plants were 3.5 to 4 feet tall at the time of inoculation. Fresh native virus extract was applied by wiping 3 leaves 6 to 10 inches long, near the top of each plant, and in addition, the virus was introduced into the stems near the growing points by means of needle punctures. The wiped leaves were numbers 20, 21, and 22, respectively, counting from the bases of the stems.

On Wisconsin-Havana Seed, many chlorotic spots appeared on all wiped leaves and, 6 days after inoculation, mosaic mottling appeared in the new leaves. No signs of local or systemic infection appeared on any of the plants of Ambalema or T.I. 448A during the test. Forty-eight days after inoculation, the T.I. 448A and Ambalema plants were beginning to set seed pods. These plants ranged from 6 to 7½ feet tall, which represented a gain of 3 feet in the length of the main stem after inoculation. At this time, 2 leaves above and 2 leaves below the points of inoculation on 4 plants of T.I. 448A and 3 of Ambalema were assayed. The data are presented in table 5.

These data show that the upper leaves collected from T.I. 448A contained

no measurable virus; the same was true in 2 of the plants of Ambalema. Downward progress in 6 of the 7 plants tested was evident. The virus was not detectable in 4 of the lowermost leaves assayed, and in Ambalema plant No. I there was no evidence of virus in any of the leaves tested. In all virus-containing leaves the virus was in very small amounts.

A second test was conducted with T.I. 448A to determine if the virus might be detected more readily from assays made on the stem cortex. During the winter all leaves on an approximately half-grown T.I. 448A plant, 32 inches tall, were wiped on their tip halves with fresh nondiluted extract of Nicotiana virus 1. Thirty-two days later, the stem was divided into lower and upper groups, the inoculated and the noninoculated portions of the leaves were batched, respectively, and prepared separately, the xylem of the stem was discarded, the cortex only being sampled. All tissues were finely pulped in a mortar and each nondiluted extract was wiped on the primary leaves of 5 bean plants. The data are presented in table 6.

TABLE 6.—*The presence or absence of Nicotiana virus 1 in different tissues of T.I. 448A tobacco when the distal halves of all leaves were inoculated on a plant 30 inches tall. Assays made 32 days after the plants were inoculated. Samples not diluted*

Sample No.	Location of tissue in the plant	Lesions per bean plant
		Number
1	Cortex from top half of stem above inoculated leaves, tip leaves included	0.0
2	Cortex from lower half of stem above inoculated leaves	0.0
3	Cortex from top third of stem in the inoculated zone	0.2
4	Proximal halves of leaves from sample 3	0.0
5	Inoculated distal halves of leaves from sample 3	181.5
6	Cortex from central third of stem in the inoculated zone	0.0
7	Proximal halves of leaves from sample 6	7.0
8	Inoculated distal halves of leaves from sample 6	342.0
9	Cortex from lower third of stem in the inoculated zone	0.0
10	Cortex from base of stem below inoculated zone	0.2

From these data it appears that the virus progressed very slowly both in leaf and stem of approximately half-grown plants, and it appears that the stem tissues do not support any appreciable increase of the virus. Even though all leaves carried considerable virus in the inoculated tip halves, practically all of it remained in those halves. It is evident that but little increase of virus occurred in tissue outside of the portions inoculated.

A third test was carried out to obtain data on the virus content of roots of middle-age and young plants of T.I. 448A and Wisconsin-Havana Seed tobacco. All plants were inoculated when very young and carried virus in the leaves at the time the roots were assayed. The roots were removed, washed thoroughly, and freed of surface water by means of soft paper. Phosphate buffer solution at pH 7 was added to the roots in double amount by weight and the mass was thoroughly pulped in a mortar. This thick liquid was assayed on 10 bean plants. The number of lesions per bean plant was as follows:

T.I. 448A:			10	weeks	old	0.0 lesions
do			15	do	do	0.3 do
Wisconsin-Havana Seed:			10	do	do	174.7 do
do	do	do	15	do	do	134.3 do

Like the stem tissues of T.I. 448A tested, the roots of this genotype carried little or no virus. In contrast, the roots of Wisconsin-Havana Seed carried considerable virus.

A fourth test was conducted with T.I. 448A inoculated at six stages of growth, beginning with plants having 7 leaves and ending with plants having about 26 leaves. All plants were from the same seedling (Table 7).

In counting the leaves, those at the base that appeared on the very small seedlings and those at the growing point that were shorter than 2 inches were disregarded. In each inoculation group one leaf near the top on each plant was wiped with nondiluted fresh virus extract from vigorous plants of Wisconsin-Havana Seed tobacco.

The plants were held until maturity. The leaves were assayed at intervals to determine the approximate end point of the virus in the plants. Well developed leaves were used for these exploratory assays because virus is slow to enter the young leaves, and rarely enters the small apical leaves. In accord with Valleau's (9) conclusions, these studies have failed to reveal the presence of virus in detectable quantities in the small central leaves of resistant tobacco plants, except in one instance when a trace was present in a leaf 3 cm. long in a young plant of T.I. 448A which had produced 10 leaves below the tested leaf, and the extracts were diluted to 10^{-4} in buffered solution.

Although the small tip leaves of infected Wisconsin-Havana Seed tobacco contain virus, tests have shown that the smallest leaves and primordial tip contain less virus than the leaves which are farther developed. Assays were carried out on leaves from the top of a plant in which mosaic had been fully established for several weeks. Leaves 12, 25, 45, 75, and 125 mm. long induced 55, 234, 276, 217, and 474 lesions per bean plant, respectively. The smallest leaf cited included the tip cluster and the stem primordium. The leaves were consecutive.

From the data in table 7 it will be noted that the end point of the virus is not sharp, especially when the plants were young when inoculated. In proceeding up the plant, the first and second leaves above the inoculated leaf had little or no virus. Each of several (10 to 20) leaves above these contained some virus. Then, in the following 6 to 8 leaves, virus was not regularly present; some leaves had none or had much less than others. Finally, in so far as could be determined by assay, the subsequent leaves were virus-free.

The progress of the virus was appreciable in young plants and it became slight to nil as the plants aged before they were inoculated.

Ordinarily, comparisons cannot be safely made between the results of assays made at different times. However, the conditions of these tests were

such that it is permissible to draw attention to the relatively high virus activity of the wiped leaves of T.I. 448A used in this test, in contrast with the low activity of the wiped leaves of the same tobacco, cited in table 4. The plants cited in table 7 were much younger, even in the last inoculation, than the plants cited in table 4. This seems to be the likely explanation for the differences in activity. In table 3 the relatively high virus activity in the basal-leaf samples from the resistant tobaccos also is explained by the fact that these plants were very young when inoculated, although in this case the basal-leaf samples did not include wiped leaves.

Virus in Relation to Side-shoots.—To determine the presence of virus in the side-shoots of plants of T.I. 448A, tests were conducted with moderately young and also with old plants. In one test 5 plants, 12 to 14 inches tall, were cut back to within one inch of the soil line. Bean plants were inocu-

TABLE 7.—*Approximate end points of virus in leaves of T.I. 448A above the inoculated leaf. Leaves numbered from base of stem*

Position of the inoculated leaf counting from the base of the stem	Position of leaf at end point of virus[a]	Approximate position of leaf at point where virus failed to appear in consecutive leaves	Lesions per bean plant from inoculated tobacco leaves[b]
No.	*No.*	*No.*	*No.*
2	30	22	296
4	26	18	239
6	23	15	.....
8	25	17	197
12	12	.....	178
24	28	[c]	120

[a] These data obtained by inoculating bean plants with leaf samples diluted in equivalent amounts of buffered solution.

[b] These extracts were diluted 200 volumes in buffered solution.

[c] There was no evidence of an appreciable increase of virus in the first three leaves above the inoculated leaf. The fourth leaf above induced only 0.3 lesions per bean plant.

lated with a nondiluted extract from the lowest cut-off leaves, and 34 lesions per bean plant were induced. Later the nondiluted extracts from the leaves of the side-shoots of the pruned plants were assayed on bean plants and 33 lesions per bean plant resulted.

In a second test, three, old, infected plants, 48 to 60 inches tall, were cut back, leaving stumps of main stem 12 inches long. One leaf was left at the top of each stump, and 2 side-shoots were allowed to grow from the 2 top buds of each stump. When the top branches were 10 to 12 inches long, the top and bottom leaves of each top shoot and the old leaves subtending them were sampled and assayed on beans. Nondiluted extracts from composited old leaves on the central axis induced 18 lesions per bean plant, but similar extracts from the leaves of the branches induced no lesions.

In a third test a part of the plants were cut off at the soil line and others 12 inches above it. When side-shoots were from 6 to 12 inches long, their

leaves and stem cortices were assayed on bean plants. All samples failed to show virus.

In a fourth test a small side-shoot with 9 leaves was found 2 inches from the soil line on an old, infected plant 50 inches tall. Assays of nondiluted extracts from the leaves of this side-shoot revealed no lesions on bean plants, whereas nondiluted extracts from the 2 lower leaves on the main stem induced 11 and 44 necrotic lesions per bean plant, respectively.

As with the virus-free leaves of the upper main stem, leaves of the side branches of T.I. 448A were found to be capable of increasing the virus. Five leaves of side-shoots on 5 infected plants were sampled by removing a lateral half of each leaf. These samples were assayed on bean plants without dilution, and found to be free from detectable virus. The halves still attached to the side-shoots were then wiped with fresh native virus extract, 11 days later, these half leaves were extracted, and, without dilution, assayed on bean plants. These extracts induced 146, 108, 11, 107, and 60 lesions per bean plant, respectively.

It is obvious from the data presented that virus did not enter side-shoots from the primary stem in measurable amounts except when the shoots developed while the infected plants were young. The leaves of the side-shoots are capable of increasing a limited amount of virus.

Tests for Starch Patterns.—Numerous tests were made to determine if the symptomless leaves of T.I. 448A that were known to carry Nicotiana virus 1 might exhibit local disturbances in starch production or starch digestion. Leaves from infected plants, wiped leaves, and virus-free leaves were collected at different times of the day, boiled in water, decolored in boiling alcohol, rinsed in warm water, and stained in the standard starch-testing solutions. In no case was it possible to detect any essential differences between leaves carrying virus and those not carrying it. When the occasional, faint, light-green areas were evident on the infected leaves, the starch-pattern test showed these spots as light areas sometimes containing light-blue centers and faint rings.

Grafting Test.—Healthy plants of T.I. 448A and Wisconsin-Havana Seed tobacco were placed with their stems in close proximity in large earthen pots. When the plants were 12 to 14 inches tall, the cortex and cambium were removed from the apposing areas of the stems at a point 6 to 8 inches above the soil line. The cut surfaces were brought in close contact with each other by means of raffia wrappings. The Wisconsin-Havana Seed plants were inoculated with Nicotiana virus 1 and all developed mosaic.

As soon as tissue unions were evident, the stems of T.I. 448A between the soil line and the union were removed. The T.I. 448A plants did not develop mosaic. Leaves from four of the most thrifty cions were assayed after dilution in 3 to 5 parts of buffered solution. The results from one typical cion showed no virus in leaves 30 and 31, traces of virus in leaves 37, 38, 42, 43 and 45, and no virus in the leaves beyond. The traces of virus induced from 0.66 to 20 necrotic lesions per bean plant. Leaves 44, 45 and

46 on the infected stock of Wisconsin-Havana Seed contained sufficient virus to induce 55.6 necrotic lesions per bean plant when diluted to 10^{-5} in buffered solution.

In comparison with tables 5 and 7, it will be observed that detectable virus was recovered higher in the cions of T.I. 448A than was the case when the direct inoculation method was used on independent plants.

DISCUSSION AND CONCLUSIONS

There seems to be little doubt that T.I. 448A is more resistant to the virus of common mosaic (Nicotiana Virus 1) than is the strain of Ambalema tobacco under study. It appears that T.I. 448A greatly reduces the virus reservoir. Failure to find appreciable amounts of virus in the roots and upper leaves, and the relatively low virus content of the lower leaves should greatly reduce the amount of virus overseasoning in these plant residues in the soil.

Chemical analyses carried out by Martin (7) and by Hills (2) indicate that the protein content in the leaves of T.I. 448A infected with Nicotiana Virus 1 is relatively low or even less than it is in leaves of virus-free plants. When the soil was maintained at a high nitrate level, the protein content of the infected leaves was slightly higher than normal, but when the nitrate level was low, the protein content of the infected plants tended to be equal to or below normal. High protein content is undesirable in commercial tobacco, and, from the data available, it appears that this factor will be of little or no consequence in infected commercial tobaccos carrying the resistance of T.I. 448A, especially in the major tobacco areas in the Southern United States, where low levels of nitrogen in the soils are maintained. It is possible that factors for the T.I. 448A type of resistance are present in the Ambalema collections.

Aside from the practical aspects of this survey, the results seem to justify the following conclusions: (1) Nicotiana Virus 1 does not move about freely in resistant plants; (2) virus rarely, if ever, enters the very young leaves of the growing tips of the resistant plants in detectable quantities, especially when the plants have advanced beyond the ten-leaf stage; (3) in T.I. 448A this virus-free zone involves from a fourth to a half of the plant at the time of maturity; (4) virus synthesis in the inoculated leaves of resistant plants tended to be less in the upper leaves of old plants than in the upper leaves of young plants, and the upper leaves of old plants tended to be more resistant than the lower active leaves, indicating that the successive leaves are increasingly resistant to virus synthesis. The basis for this resistance is not known. When leaves are inoculated by wiping, the number of vulnerable points for entry and the susceptibility of these points must be taken into consideration.

The evidence obtained in the grafting test indicates that the virus did move farther in T.I. 448A cions on infected Wisconsin-Havana Seed plants, than was the case when young T.I. 448A plants were inoculated directly.

When one considers the large quantities of virus in the susceptible Wisconsin-Havana Seed plants, this observation is not surprising. However, it is clearly evident from the data that little virus reached the upper leaves of the resistant cions.

This observation and the results from the direct inoculations suggest that the channels of virus flow are restricted in some manner in T.I. 448A. On the other hand, it is quite possible that these channels in resistant plants are in no way different from those in susceptible plants. If this is true, it would appear that the distance which the majority of virus particles travel in the plant tends to be limited to a considerable degree, and that movement over long distances is accomplished, for the most part, by stages between zones of synthesis and by relatively few particles that get into the main vascular channels. After the virus enters the growing-point zone of the stem in highly susceptible plants, the problem of movement into all subsequent leaves is greatly reduced, in comparison with the situation in resistant plants whose growing points seldom, if ever, receive virus from the zones of synthesis.

Holmes (3), working with common mosaic in Turkish tobacco, a very susceptible type, presented data which make it appear that the bulk of the virus tends to remain in or very near the infection sites for a long time, a small amount trickling out to start new infection sites. If a similar situation obtains in T.I. 448A, with its low level of virus synthesis, it is evident that the amount of virus moving out of the infection sites gradually approaches the zero point as infection proceeds in the increasingly resistant leaves of the growing plant, and in the test cited in table 5, the more rapid progress of the virus down the plant may have been facilitated by the apparently greater susceptibility of the leaves as we proceed in that direction (Table 4).

Resistant tobacco is of value for studies on virus movement, but further study will be required to determine if the results obtained with resistant plants can be applied directly to highly susceptible plants.

To the student of gross pathology, T.I. 448A falls in the class of so-called symptomless carriers, and is immune from the mosaic disease. However, from the standpoint of those who are concerned with the solution of the basic problems encountered in resistance and immunity, the interest must go beyond gross symptoms. These investigators must concern themselves with the minor as well as the major signs of disease in resistant and in highly susceptible genotypes. When the problem is approached in such a manner, it is difficult to escape the conclusion that natural immunity should be determined primarily on the basis of nonsupport of a pathogen rather than on a basis of symptoms. In T.I. 448A, the virus induces slight changes (2) in the oxidase, catalase, peroxidase and protein, but as slight as these changes are, they do represent signs of disease. As indicated in an earlier note (5), it seems unlikely that any so-called symptomless carrier is totally free from signs of disease.

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GROWTH OF PHYTOPATHOGENIC BACTERIA IN A SYNTHETIC ASPARAGIN MEDIUM

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(Accepted for publication August 8, 1942)

During an investigation of the nutrition of phytopathogenic bacteria, it became necessary to use a variety of synthetic media. In this connection, experiments were performed to determine for the growth of the bacterial phytopathogens the suitability of a medium containing asparagin as the sole source of both carbon and nitrogen.

Use of synthetic asparagin media is not new to bacterial plant pathology. Smith (14, 15) records several cases where asparagin sufficed as the nitrogen source for phytopathogenic bacteria. Clara (4) reports that the 19 species of green-fluorescent pigment-producing plant pathogenic bacteria that he tested, grew in a medium containing asparagin as the only carbon and nitrogen source. Burkholder (2) states that the members of the yellow-pigmented "*Phytomonas campestris* group" do ". . . not utilize asparagin as a source of carbon and nitrogen, as the green fluorescent [phytopathogenic *Pseudomonas*] forms do . . . " and, in a personal communication, explains that this statement is based on unpublished observations he had made of the growth of these species in Clara's (4) asparagin medium. As has been noted (1, 2, 3, 6, 7, 16), the genus *Phytomonas* Bergey *et al.* is composed of several unlike groups of phytopathogenic bacteria. Hence, it appeared likely that confirmation of the foregoing and an extension that would include representatives of all the different groups comprising that outmoded genus, would aid in establishing the validity of the groupings that have been recommended (2, 6, 7, 16).

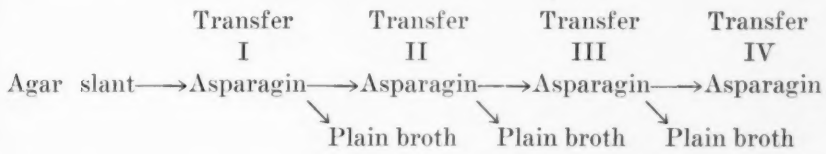
EXPERIMENTAL

One hundred and seventy-three isolates of plant-pathogenic bacteria, consisting of 66 species and varieties, were tested for their ability to grow when transferred repeatedly in a synthetic asparagin medium. In the main, these isolates are the same as those cultured by Starr and Burkholder (16) in their studies on lipase activity; reference to that publication will give additional information concerning each culture.

The medium used in these experiments consisted of 0.1 per cent KH_2PO_4, 0.02 per cent KCl, 0.02 per cent $MgSO_4 \cdot 7H_2O$, 0.5 per cent asparagin, pH 6.4. In preparing this medium, a suitably concentrated solution of the inorganic salts was tubed and sterilized by autoclaving. To this was added aseptically the proper amount of a concentrated solution of asparagin, sterilized by filtration through an L5 Chamberland candle. The resulting medium was incubated before use to assure sterility.

Throughout this investigation, all glassware was cleaned by soaking in dichromate-sulphuric acid cleaning solution for at least 24 hours, washing thoroughly in tap water, and then rinsing several times in distilled water. Careful cleaning is essential in order to eliminate the minute quantities of organic matter that ordinary washing does not remove and which might be used by the bacteria in lieu of asparagin.

Actively growing cells from agar slant cultures were transferred to the synthetic asparagin medium. To avoid contamination of the synthetic medium with unknown substances that might support growth instead of the asparagin, the inocula were kept small and care was taken to carry over cells and not agar. It is inevitable, however, that certain metabolites associated with the cells will be introduced; these might account for growth in the first transfer. Since it is the opinion of experts in the field of bacterial nutrition (9, 10) that this added material can be eliminated by several successive transfers in the medium, the asparagin cultures were transferred serially by wire loop to fresh asparagin at 4- to 6-day intervals. To prove that failure to grow in the asparagin medium is to be attributed to the insufficiency of this medium rather than to dead inocula, transfers usually were made at the same time to plain nutrient broth as follows:



In all cases, the organisms grew in these nutrient broth controls.

All cultures were incubated at 27° C. and examined daily for growth. Growth was defined as a distinct turbidity within 6 days after inoculation and was checked by gram staining the last culture in each series. The results of these experiments are presented below.

For reasons discussed in detail elsewhere (2, 16), the generic names of the species listed in the following paragraphs have been changed from *Phytomonas* to those now considered more appropriate; *viz.*, *Xanthomonas* Dowson (6) for the members of the yellow-pigmented "*Phytomonas campestris* group" (2); *Pseudomonas* Migula for the green-fluorescent pigment-producing and related phytopathogenic bacteria (4, 6, 12); *Agrobacterium* Conn (5) for the members of the tumor-forming "*Phytomonas tumefaciens* group"; *Corynebacterium* Lehmann and Neumann for certain of the gram-positive forms (7, 8, 13, 17); *Phytomonas* Bergey *et al.* is retained for those phytopathogenic bacteria still of doubtful systematic position.

The notation after each name is the designation now used for the isolate in the stock-culture collections of W. H. Burkholder, Cornell University, and of the Bacteriological Laboratory at Brooklyn College. The number in parenthesis refers to the designation used for that culture by Starr and Burkholder (16).

The phytopathogenic bacteria that grew through 4 successive transfers in the synthetic asparagin medium are: *Agrobacterium gypsophilae* TG1 (NB), *A. radiobacter* TR1 (H), *A. savastanoi* TS3 (COS), *A. savastanoi* var. *fraxini* TS1 (NB), *A. tonellianum* TT1 (COS), *A. tumefaciens* TT2 (B2); TT3 (B6), *Corynebacterium fascians* CF1 (R); CF2 (S), *Phytomonas stewartii* SS2 (S16); SS3 (S15); SS6 (B93); SS8 (B95); SS9 (B96); SS11 (3152); SS12 (3153); SS13 (3154), *Pseudomonas alliicola* PA5 (4); PA6 (1), *P. angulata* PA3 (B99); PA11; PA12, *P. atrofaciens* PA13; PA14, *P. berberidis* PB3, *P. caryophylli* PC3 (3); PC8 (R8), *P. cichori* PC1 (G18), *P. coronafaciens* PC17; PC18, *P. delphinii* PD1 (G36), *P. glycinea* PG1; PG2; PG3; PG4; PG5; PG6; PG7, *P. jaggeri* PJ1, *P. lachrymans* PL1 (G12); PL3, *P. lapsa* PL2 (Ark), *P. maculicola* PM1 (G37), *P. medicaginis* var. *phaseolicola* PM3 (G3); PM4 (G31); PM5 (G29); PM7, *P. mellea* PM8, *P. papulans* PP4, *P. pisi* PP1 (G32), *P. polycolor* PP2 (G11), *P. primulae* PP3 (Ark), *P. solanacearum* PS14; PS15, *P. striafaciens* PS1 (70), *P. syringae* PS2 (B38); PS3 (B45); S4 (B70); PS5 (SyF); PS6 (G21); PS7 (G28); PS8 (G24); PS9 (Syl); PS11 (G20); PS12 (G19); PS13 (G7), *P. tabaci* PT1; PT3; PT4; PT5; PT6, *P. tomato* PT7; PT8, *P. viburni* PV1 (1); PV3 (3), *P. viridiflava* PV5 (G5), *P. viridilivida* PV4 (G23).

The phytopathogenic bacteria that did not grow in the synthetic asparagin medium are: *Agrobacterium rubi* TR2; TR3, *Corynebacterium flaccumfaciens* CF3[a] (S1); CF6 (S4); CF8[a] (S6); CF9 (S7); CF10 (S9); CF11 (S20); CF12 (S31), *C. insidiosum* C11 (S23); C12 (S21); C13 (S34), *C. michiganense* CM1 (S29); CM2 (S18); CM3 (S30); CM4 (S11), *C. poinsettiae* CP1; CP2; CP3; CP5; CP13; CP14; CP20; CP21; CP27; CP31; CP35; CP37; CP39; CP41, *Phytomonas manihotis* SM1 (R2); SM2 (R3); SM3 (R4); SM5 (4); SM6 (R1); SM9, *P. mors-prunorum* PM6 (B69), *P. stewartii* SS1[a] (S13); eight isolates *do* grow (*cf.* preceding list and text), *P. tardicrescens* ST1 (S26), *Xanthomonas barbareae* XB1 (B1); XB2 (BR), *X. begoniae* XB3 (96); XB6, *X. campestris* XC1 (85); XC2 (86); XC3 (R4), *X. campestris* var. *armoraciae* XC4 (HI), *X. corylina* XC5[a] (78), *X. geranii* XG1 (2); XG2 (3); XG3 (5); XG4 (G1); XG5 (87); XG6 (89), *X. gummisudans* XG7 (94), *X. hederae* XH1 (16), *X. incanae* XI1 (St1); XI2 (St2); XI3 (St3), *X. juglandis* XJ1 (77); XJ2 (79), *X. malvacearum* XM1[a] (1); XM2[a] (B87), *X. papavericola* XP5 (47), *X. pelargonii* XP6 (92); XP7[a] (93); XP8[a] (100), *X. phaseoli* XP1 (H); XP2 (B16); XP4 (E2); XP14 (MPS), *X. phaseoli* var. *sojense* XP3 (11), *X. pruni* XP9[a] (B65); XP10[a] (1); XP11[a] (2); XP12[a] (3); XP13[a] (B67), *X. translucens* XT1 (90), *X. translucens* f. sp. *hordei-avenae* XT6, *X. translucens* var. *undulosa* XT4 (91); XT5 (83), *X. vasculorum* XV1[a] (49), *X. vesicatoria* XV3 (1); XV5 (3); XV6 (4); XV7 (5); XV8 (6); XV9 (7); XV10 (8); XV11[a] (13); XV12 (38); XV13 (L6); XV14 (99); XV15[a] (VI), *X. vesicatoria* var. *raphani* XV16 (L11), *X. vitians* XV2 (14).

[a] This isolate sometimes forms a scanty turbidity in the first transfer to the synthetic asparagin medium, but does not grow at all on subsequent transfers in this medium. Moreover, the quantity of growth, in these cases, is less than that produced by the organisms listed in the preceding paragraph.

DISCUSSION

The data presented in the foregoing show that the members of the outmoded genus *Phytomonas* Bergey *et al.* vary in ability to grow in a medium containing asparagin as their only source of both carbon and nitrogen. It should, however, be recognized that certain of the groups comprising this genus act characteristically in this medium: all 60 isolates of phytopathogenic *Pseudomonas* grow well on repeated transfer in the synthetic medium, while none of the 57 *Xanthomonas* isolates can do so. This confirms the work of Clara (4) and of Burkholder (2) mentioned above. It must be noted, however, that some of the *Xanthomonas* isolates do product a slight turbidity in the first transfer in the synthetic asparagin medium. This growth probably occurs at the expense of nutrients carried over with the inocula, which are frequently heavy in this group because of the slimy nature of the growth on agar. There is no growth of any of the *Xanthomonas* species on subsequent transfer in the asparagin medium and, moreover, the scanty turbidity produced by these species in the first transfer at no time approaches in quantity that made by the *Pseudomonas* isolates.

The genus *Agrobacterium* was recently established in the Rhizobiaceae by Conn (5) to include the gall-forming phytopathogenic bacteria and related soil forms. Isolates of the type species, *Agrobacterium tumefaciens*, as well as of *A. radiobacter*, *A. tonellianum*, *A. gypsophilae*, and *A. savastanoi* grow well in the synthetic asparagin medium. Accordingly, it is interesting that the cane gall organism, *A. rubi*, which is otherwise closely related to *A. tumefaciens*, cannot grow at all in the asparagin medium. Relevant to this is Pinckard's (11) observation that *A. rubi* grew only slightly in a glucose-asparagin medium in which other members of this group grew well.

When one considers the relatively complex nutritive requirements of *Corynebacterium diphtheriae* (10), it is not surprising that the phytopathogenic *Corynebacterium* species fail to grow with asparagin as the only source of carbon and nitrogen. The only exception is *C. fascians*, which grows rapidly and luxuriantly in this medium; perhaps this species is not now classified correctly.

Dowson (6) placed the corn-wilt bacterium, *Phytomonas stewartii*, in the genus *Xanthomonas*, although Burkholder had excluded it from the equivalent "*Phytomonas* proper" group (3). This organism possesses certain characteristics which differentiate it from most species of *Xanthomonas*, *viz.*, lack of flagella (3, 15), failure to peptonize milk (3, 15) or to produce hydrogen sulphide, inability to attack cottonseed oil as shown by the spirit blue agar technique (16). To these differences, there now can be added the ability of *P. stewartii* to grow in a medium in which asparagin is the sole carbon and nitrogen source, as contrasted with the inability of typical *Xanthomonas* species to grow under identical conditions. This asparagin medium is not completely suitable for the maximal development of *P. stewartii* since growth was rather slow and, indeed, one atypical isolate (SS1) did not grow after the first transfer. However, some significance

must be attached to the fact that 8 of the 9 *P. stewartii* cultures tested produced a distinct growth through 4 successive transfers in the synthetic asparagin medium while no typical *Xanthomonas* isolate ever did. For the aforementioned reasons, it is recommended that the corn-wilt bacterium be excluded from *Xanthomonas* and that the name *Phytomonas stewartii* be retained until such time as the exact taxonomic position of that species be determined.

SUMMARY

A series of 173 isolates of phytopathogenic bacteria, consisting of 66 different species and varieties, was tested for the ability to grow through 4 successive transfers in a synthetic medium that contained asparagin as the only source of both carbon and nitrogen. Certain of the genera and groups comprising the outmoded genus *Phytomonas* Bergey *et al.* have a distinctive action on this medium. Accordingly, this characteristic may be valuable in classifying the bacterial plant pathogens.

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A BOTRYTIS DISEASE OF LUPINES[1]

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INTRODUCTION

For the past few years 3 species of imported lupines (*Lupinus angustifolius*, *L. albus* and *L. luteus*) have been grown experimentally in the Southeastern United States, and *L. angustifolius* has become fairly well established as a winter cover crop. Owing to their lack of winter hardiness lupines can be grown only over a very limited area and, even then, they may be severely injured in cold winters. In February, 1938, following a January freeze, which injured some lupine plantings severely, a disease was quite abundant at Tifton, Georgia, and to a less extent at Gainesville, Florida. Whether the disease was actually responsible for the dying of the plants or was simply following the freezing injury was not clear. The nature and severity of the disease, however, seemed to justify investigation, and it is the purpose of this paper to record the consequent results.

SYMPTOMATOLOGY

Large plants, some already in bloom, showed all degrees of injury. Some had been dead for a considerable period, others were dwarfed, and still others had made good growth and then were being partly or entirely killed. An examination showed that cankers, sometimes several inches long, in the main stem near the soil or at different distances above, were girdling the stem and causing death of the part above. In some instances only a portion of the top or small branches were dying. The cankers usually, if not always, had a small infected or dead stem at or near the center, suggesting that this was the point of attack that resulted in the death of the part affected. Sometimes, leaflets, petioles, or stipules, were involved. Stem girdling eventually resulted in the wilting and death of the parts above. When a leaflet or other small organ was attacked, it died rapidly, and the petiole or branch and often the large branches or the main stem itself became involved and killed. Something of the nature of the injury is apparent in figure 1, A. Here are shown 2 large plants, the one at the right is healthy and that on the left is wilting and the leaves drooping because of a large canker near the ground, not evident in the picture. The cankers are usually greenish to brown and may be smooth or have a grayish coating of fungous mycelium and conidiophores. The nature of the cankers is shown in figure 1, C, and an enlargement of the same cankers after being held in the moist chamber for 4 days is shown in figure 1, D. The stem at the left in figure 1, C, shows small dead branches that served as the avenue through which

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 100, Journal Series, Georgia Agricultural Experiment Station.

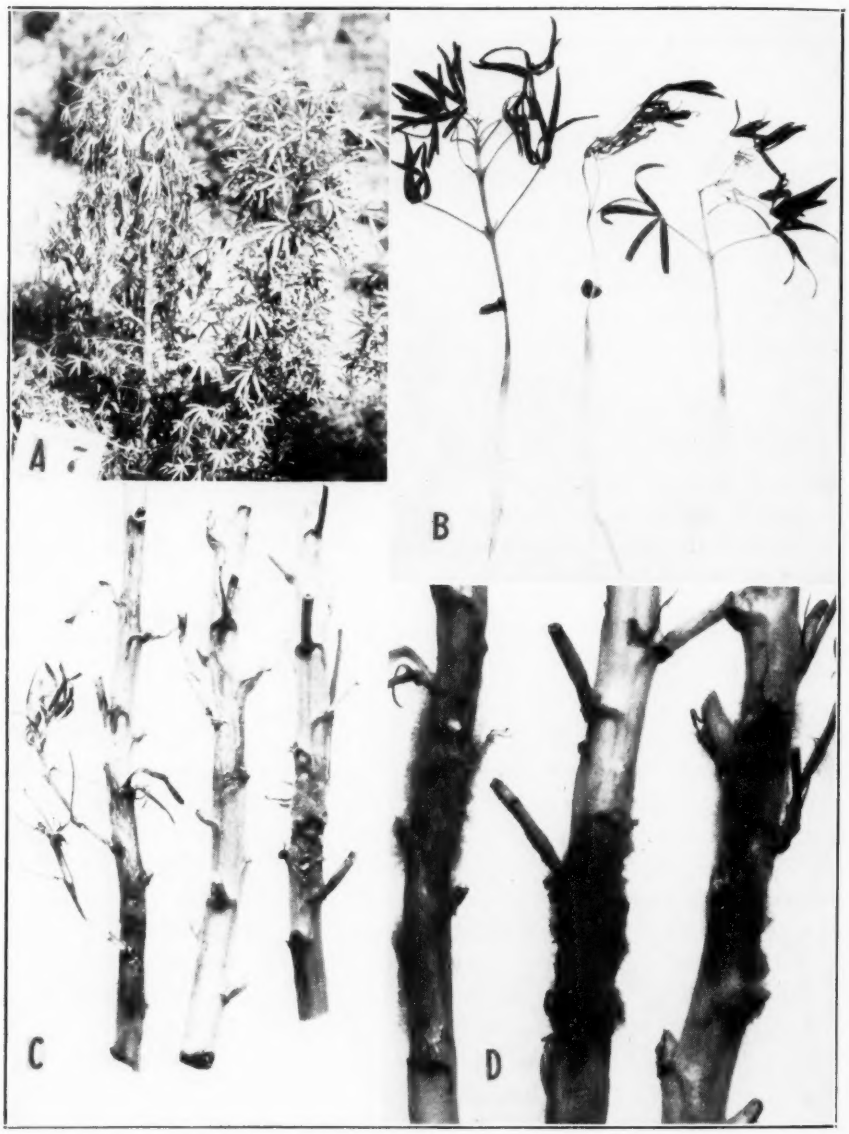

FIG. 1. *Botrytis cinerea* on *Lupinus angustifolius*. A. Two large lupine plants growing in a field at Gainesville, Fla. The plant at the left shows wilting of the leaves and bending over of the terminal blossom cluster due largely to girdling of the stem. In a few instances separate infections had occurred in the young leaflets at the ends of branches. The plant at the right is healthy. $\times \frac{1}{12}$. B. The 2 young lupine plants at the left have been infected through the cotyledons. The center plant also had some infected leaflets. The plant at the right was infected through very young leaflets near the terminal bud. $\times \frac{1}{5}$. C. The canker at the left was caused by infection of the stem through the side branches. The stem at the right shows some compact fungus growth, such as is sometimes seen during medium dry periods. Often no fungus is apparent on the surface of the canker. $\times \frac{1}{2}$. D. Such cankers as in C covered with the aerial mycelium and conidiophores of the *Botrytis* after 3 or 4 days of wet weather. $\times 12/13$.

the larger stem was invaded. Similar dead twigs are almost always present in the cankers. The stem at the right shows the fungus fruiting on the surface as it does under moist conditions, such as during a rainy period. A clearer view of what these cankers look like during a wet spell is shown in figure 1, D. Here the fungus has come out of the tissue and fruited over the surface of the lesion.

ETIOLOGY

A study of the fungus fruiting on the surface of the lesions and isolated from the inner tissues showed that it was a species of *Botrytis*. Inoculations were made to determine whether this fungus was able to attack healthy plants or simply followed into frozen tissue. Field evidence seemed to support both possibilities. The disease is seen in the field, at least, more commonly following a cold winter. Nevertheless there seemed to be instances where it was attacking young twigs that had developed since any frost had occurred.

Inoculation experiments were made in which a heavy suspension of spores from a pure culture of the fungus in tap water was atomized onto 10 seedlings of *Lupinus angustifolius* that varied in size from just out of the soil to 3 inches tall. The terminal buds of some plants were not yet exposed, while the first true leaves of other plants were fairly well-developed. The plants were held at laboratory temperature (60–75° C.) under bell jars for 72 hours. No infection resulted in any of the plants. The cause for the total lack of infection was not evident but it was thought that the mean temperature might have been too low.

Since *Botrytis* belongs to a group of fungi some of which need a saprophytic start before being able to invade healthy tissue, the above experiment was repeated but with bits of inoculum (hyphae) with or without agar attached. The mycelial felt was broken up into small pieces in water and applied to the plants with a brush. Four days after these inoculations were made 3 plants out of 9 showed infection. The fungus grew from the pieces of inoculum into the tissue and caused a rapid decay of the invaded parts. In some cases petioles were entirely decayed through and the leaves wilted; in other instances the fungus had entered the plant through the leaf axil; in still others the cotyledons were invaded. In one pot 5 out of 10 plants were infected. Infection took place through the leaf blade, petiole, stipule, terminal bud, or cotyledons, but not through the main stem. The latter was invaded and killed, but only after the fungus had first attacked some young tender part serving as a point of entry. Figure 1, B, shows 3 of the inoculated plants. The 2 at the left have been invaded through the cotyledons, while the terminal bud of the plant at the right was killed. The fungus was reisolated from the decayed tissue and its parasitism again proved. None of the control plants were attacked.

These experiments show that *Botrytis* can attack young healthy plants and produce symptoms similar to those seen in the field. No freezing or

other injury is necessary. It is believed, however, that a high humidity is essential and it also seems probable that infection can take place, more readily at least, if some provision is at hand to permit the fungus to get a saprophytic start. For example, pieces of infected tissue broken from a diseased plant might become lodged in the axil of a leaf and under conditions of high humidity the fungus grow from the diseased into the healthy tissue. It is possible that spores might fall onto a part of a healthy plant where dirt or organic matter has accumulated that would provide the necessary food for the beginning of active growth. The fungus also may find suitable conditions for infection in the organic matter at or near the surface of the soil. Frozen tissue, under conditions suitable for the rapid development of the fungus, doubtless provides an ideal infection court. This accounts for the abundance of the disease following a freeze when the fungus can attack many parts of the plant and greatly augment the damage done by low temperature.

The causal fungus is easily isolated and grows well on all media tried by the writer. On potato agar with 2 per cent dextrose added, there develops a rather dense grayish mat, nearly black next to the medium in the lower half of the slant. Little or no fruiting occurs on this medium. On oatmeal agar the fungus forms a loose hyphal growth instead of the dense mat, as on potato dextrose agar. A large part or all of the space between the agar slant and the wall of the tube usually is filled with the loose grayish mycelium, similar to that seen on the cankers during wet weather. The fungus, ordinarily, does not sporulate abundantly on culture media. It fruits fairly well, however, when grown on oatmeal agar on a long slant in a large test tube. The spores range from 8.4–13.65 × 6.8–10.5 μ (average of 50 spores 10.6 × 8.2 μ). The mycelium on oatmeal agar ranges from 8 to 12.6 μ in diameter.

A study of the fungus leaves little doubt that it belongs in the *Botrytis cinerea* group.[2] It has the typical cinerea type of conidiophore. Irregular, mid-size, black sclerotia are produced rather sparingly in culture and probably in or on the host, although they were not seen on the latter. These sclerotia, no doubt, serve to carry the fungus over from one crop season until the next. No apothecial stage has been seen.

What appears to be the same disease is described by Curtis[3] in New Zealand, by Dippenaar[4] in South Africa, and by Pape[5] in Germany. The disease probably is coextensive with the crop.

CONTROL

As is the case of diseases caused by other soil-borne fungi, the botrytis

[2] This conclusion was confirmed by H. H. Whetzel, Cornell University, Ithaca, N. Y., who kindly examined cultures of the fungus.

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disease of lupines is difficult to control. Certain rather obvious precautions may aid in reducing losses. Some of these are: practice rotation, avoid wet areas, do not plant seed too thick, use only the hardiest varieties available, and do not attempt to grow the crop too far north until more winter-hardy selections are available.

SUMMARY

A disease of lupines caused by a fungus of the *Botrytis cinerea* group is described. This disease characteristically produces cankers on the stems or branches, often girdling them and killing the parts above. Although not essential for infection, frozen tissue forms an excellent infection court and damage caused by late spring freezes may be considerably augmented by the fungus.

PHYSIOLOGIC RACES OF USTILAGO NIGRA[1]

V. F. TAPKE

(Accepted for publication June 16, 1942)

Ustilago nigra Tapke, described in 1932 as a seedling-infecting loose smut of barley (2), is now apparently as widespread and damaging in the United States as *U. nuda* (7), the well-known floral-infecting loose smut of barley. The estimated annual loss caused by these smuts (7) amounts to 2,000,000 bushels, a loss that now may be halved through seed treatment with certain easily applied surface disinfectants, as *U. nigra* is readily controlled by these chemical treatments (2, 3, 7). The problem of control, however, may be greatly simplified through the use of varieties resistant to both *U. nuda* and *U. nigra*. To aid in breeding for resistance against *U. nigra*, a study of physiologic races in this species, therefore, was begun. The present report embraces the results of the 7-year period, 1935–1941.

EARLIER RESEARCH

Two physiologic races in *Ustilago nigra* were first reported in 1936 (4). In a recent abstract, Josephson (1) notes the occurrence of 5 distinct races and possibly 3 others in a barley loose smut referred to as *U. medians* (*U. nigra*) (6, 7).

MATERIALS AND METHODS

The experiments reported herein were conducted at Ithaca, New York, in 1935 and from 1937 to 1941, inclusive.[2] The technique was the same as that described in a study of physiologic races of covered smut of barley (*Ustilago hordei*) (5). The differential hosts were pure lines of Excelsior (C.I. 1248), Hannchen (C.I. 531), Himalaya (C.I. 1312), Lion (C.I. 923), Nepal (C.I. 595), and Odessa (C.I. 934). One hundred and sixty-eight collections of *U. nigra* were tested, including the 10 studied in 1935 on which a previous report was made (4). The percentages of smut were based on counts of 300 heads per row in duplicate seedings each year.

As the different races were isolated, a type collection of each was selected and continued in the later tests. The inoculum of each race was collected from the variety that most clearly differentiated it from others. The different races were identified by the general resistance or susceptibility of the differential varieties.

EXPERIMENTAL RESULTS

Seven distinct physiologic races were isolated in the study of the 168 collections of *Ustilago nigra*. The reaction of a type collection of each race,

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the New York (Cornell) Agricultural Experiment Station.

[2] For supervision of the field plantings at Ithaca, and for other helpful assistance, the writer is indebted to Dr. H. H. Love and Mr. W. T. Craig, Department of Plant Breeding at Cornell University.

beginning with the year in which it was isolated, is presented in table 1. Races 4 and 5 are the two described in 1936, but not numbered (4). The results show consistent differences in the pathogenicity of the different races. It is evident that conditions for smut were better in some years than in

TABLE 1.—*Percentages of loose smut produced on six differential varieties by seven physiologic races of Ustilago nigra*

Race No.	Year	Smutted heads in						Race identified mainly by the susceptibility of
		Excelsior C.I. 1248	Hannchen C.I. 531	Himalaya C.I. 1312	Lion C.I. 923	Nepal C.I. 595	Odessa C.I. 934	
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
1	1937	0.0	0.0	0.0	0.0	0.0	4.0	Odessa
	1938	0.0	0.0	0.0	0.0	0.0	36.0	
	1939	0.0	0.0	0.0	0.0	5.0	65.0	
	1940	0.0	0.0	0.0	0.0	0.0	42.0	
	1941	0.0	0.0	0.0	0.0	0.0	29.0	
2	1937	0.0	12.0	0.0	0.0	0.0	24.0	Hannchen and Odessa
	1938	0.0	42.0	0.0	0.0	0.0	53.0	
	1939	0.0	51.0	0.0	0.0	0.0	65.0	
	1940	0.0	24.0	0.0	0.0	0.0	49.0	
	1941	0.0	15.0	0.0	0.0	0.0	17.0	
3	1938	0.0	0.0	0.0	20.0	0.0	55.0	Lion and Odessa
	1939	0.0	1.0	0.0	17.0	0.0	81.0	
	1940	0.0	0.0	0.0	9.0	0.0	57.0	
	1941	0.0	0.0	0.0	4.0	0.0	33.0	
4	1935	0.0	33.0	0.0	37.0	0.0	60.0	Hannchen, Lion and Odessa
	1937	0.0	20.0	0.0	16.0	0.0	55.0	
	1938	0.0	29.0	0.0	19.0	0.0	62.0	
	1939	0.0	46.0	1.0	24.0	2.0	82.0	
	1940	0.0	33.0	0.0	22.0	0.0	55.0	
	1941	0.0	30.0	0.0	17.0	0.0	35.0	
5	1935	0.0	16.0	17.0	3.0	32.0	48.0	Hannchen, Himalaya, Lion, Nepal and Odessa
	1937	0.0	29.0	28.0	8.0	43.0	39.0	
	1938	0.0	9.0	19.0	13.0	20.0	45.0	
	1939	0.0	28.0	36.0	15.0	54.0	70.0	
	1940	0.0	25.0	28.0	11.0	21.0	52.0	
	1941	0.0	8.0	10.0	3.0	5.0	18.0	
6	1937	21.0	8.0	13.0	9.0	35.0	28.0	All six varieties
	1938	16.0	7.0	16.0	16.0	14.0	30.0	
	1939	44.0	19.0	22.0	7.0	53.0	58.0	
	1940	30.0	12.0	19.0	11.0	22.0	44.0	
	1941	7.0	3.0	6.0	3.0	6.0	18.0	
7	1937	0.0	26.0	23.0	0.0	27.0	40.0	Hannchen, Himalaya, Nepal and Odessa
	1938	0.0	13.0	12.0	0.0	22.0	30.0	
	1939	0.0	47.0	16.0	0.0	29.0	65.0	
	1940	0.0	9.0	12.0	0.0	28.0	35.0	
	1941	0.0	11.0	20.0	0.0	26.0	24.0	

others. In 1939, for example, infection percentages ran much higher than in 1941. Despite this variability, however, the identity of each of the 7 races was clearly maintained. Under the favorable conditions of 1939 some varieties, such as Nepal, were, that year only, lightly smutted by certain races.

The frequency of occurrence and the distribution by States of the 7 races of *Ustilago nigra* are presented in table 2. It will be noted that race 4 was collected more often than all of the others combined, and that it occurred over wide areas of the United States. Of interest is the fact that in a study of physiologic races of *U. hordei* (5), the writer likewise isolated a widespread race (race 6), which occurred 114 times in 200 collections. This, like race 4 of *U. nigra,* also is characterized by the susceptibility of

TABLE 2.—*Frequency of occurrence and distribution by States of physiologic races of Ustilago nigra in 168 collections from 23 States*

Location	Collections of race number							Total collections from the State
	1	2	3	4	5	6	7	
Arkansas	3	...	1	1	...	...	...	5
Colorado	...	...	...	4	...	...	...	4
Delaware	1	...	...	...	...	...	...	1
Illinois	2	2	...	...	...	...	...	4
Indiana	3	...	...	...	...	...	...	3
Iowa	...	2	...	9	...	...	...	11
Kansas	...	1	...	...	...	1	...	2
Kentucky	1	...	...	...	...	...	...	1
Maryland	2	...	...	...	...	...	...	2
Minnesota	...	2	...	18	4	5	2	31
Missouri	2	1	...	1	...	...	...	4
New York	1	3	...	11	...	...	...	15
North Carolina	1	...	...	...	...	...	...	1
North Dakota	...	...	...	2	...	...	...	2
Ohio	...	...	...	1	...	...	...	1
Oklahoma	1	...	...	...	...	...	...	1
Pennsylvania	12	3	...	1	...	...	...	16
South Dakota	...	...	...	3	1	2	...	6
Tennessee	3	...	1	...	...	...	...	4
Texas	...	1	...	16	...	...	...	17
Virginia	8	...	...	2	...	...	...	10
Washington	...	...	...	2	...	...	...	2
Wisconsin	...	...	...	15	2	7	1	25
Total collections of each race	40	15	2	86	7	15	3	168

Hannchen, Lion, and Odessa and by the resistance of Excelsior and Nepal[3] to its attack. Also, *U. nigra* races 1, 2, and 3 produced reactions similar to those of *U. hordei* races 8, 1, and 5, respectively, on the varieties common to both tests.

PATHOGENIC STABILITY OF RACES

The data in table 1 show that the 7 collections typifying the races of *Ustilago nigra* were pathogenically stable throughout the years they have been studied. The remaining 161 collections tested in 1 or 2 years only, also produced such clear-cut reactions that there was no question concerning their race allocation. Two of three other collections tested, but not included here, were evidently mechanical mixtures of several races. The other, a

[3] Himalaya (C.I. 1312) was not used as a differential host in the earlier covered smut tests (5) but in recent years this variety has proved immune from *U. hordei* race 6, as well as *U. nigra* race 4.

collection from Missouri, may deserve classification as an eighth race of *U. nigra,* but the reaction of Excelsior to it has been variable.

SUMMARY AND CONCLUSIONS

Seven distinct races of the seedling-infecting barley loose-smut fungus, *Ustilago nigra,* were found in 168 collections of that species from 23 States. The frequency of occurrence of the races and their distribution by States are tabulated.

Race 4 occurred more frequently than all the others combined. This race is identified by the same reaction of the differential varieties that identified a previously described race (race 6) of barley covered smut, which also occurred in over half of the collections of that smut in an earlier study.

In breeding for resistance against *Ustilago nigra,* as with other small grain smuts, the existence of physiologic races must be taken into account.

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PHYTOPATHOLOGICAL NOTES

An Unusual Sporophore of Trametes suaveolens Produced on Artificially Inoculated Wood.[1]—In a preliminary study to determine the effect of environment on the gross morphology of fruit bodies and to compare those produced artificially with those found in nature, cultures were made as follows.

Two sections 7 inches long and $2\frac{1}{2}$ inches in diameter were cut from fresh green branches of green ash, *Fraxinus pennsylvanica*, var. *lanceolata* Sarg., and soft maple, *Acer saccharinum* L. A hole $1\frac{1}{4}$ inches in diameter and 6 inches deep was bored in each and filled to within one inch of the top with a mixture of wheat and oats that had been autoclaved at 15 pounds' pressure for 1 hour on 2 successive days. Corks were placed loosely in the holes. The two pieces were then placed vertically in a gallon jar containing about 500 cc. of water, and the cover was screwed on lightly. The jar and contents were autoclaved for 1 hour at 15 pounds' pressure and allowed to cool in the autoclave.

When cool, the grain mixture was inoculated with a culture of *Trametes suaveolens* (L.) Fries isolated from a sporophore found on a maple stump near St. Paul, Minnesota, in November, 1940. Immediately after inoculation (Jan. 2, 1941) the holes in the wood sections were tightly plugged with corks and the jar cap tightened. The culture was kept in the laboratory.

After about 8 weeks mycelium was noticed growing out between the corks and the wood, and by May 1, a thick mycelial mat covered both pieces of wood. The quantity of water originally placed in the jar increased rather than decreased during this time, apparently being augmented by water released by the fungus during the decay process. A few rounded humps of mycelium formed near the upper end of the wood, but no definite fruiting primordia appeared. On August 13 the wood pieces, by this time bound firmly together by mycelium, were placed in a flat of moist sand beneath the north side of a greenhouse bench, the bottom ends being buried about 3 inches in the sand. Tap water was added to the sand occasionally. Although not exposed to direct sunlight, the specimens were located where the light was sufficient to permit the apparently normal growth of various green plants.

About September 1 shallow pores were noticed on the surface of one of the humps of mycelium, and by November 1 the compound sporophore (Fig. 1, A and B) had attained its final size, being approximately 4 inches wide. It was photographed, and agar cultures were made from it, on December 1, 1941.

The pores of the original fruit body are shown in figure 1, C, and those of the largest pileus in the compound sporophore produced in the greenhouse are shown for comparison in figure 1, D. Whether the obvious deviations from the normal in the general form of the sporophore and in the size,

[1] Paper No. 2031 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

shape, and position of the pores of the latter can be ascribed to the substrate on which the fungus grew or to light or humidity, we cannot say. The wood

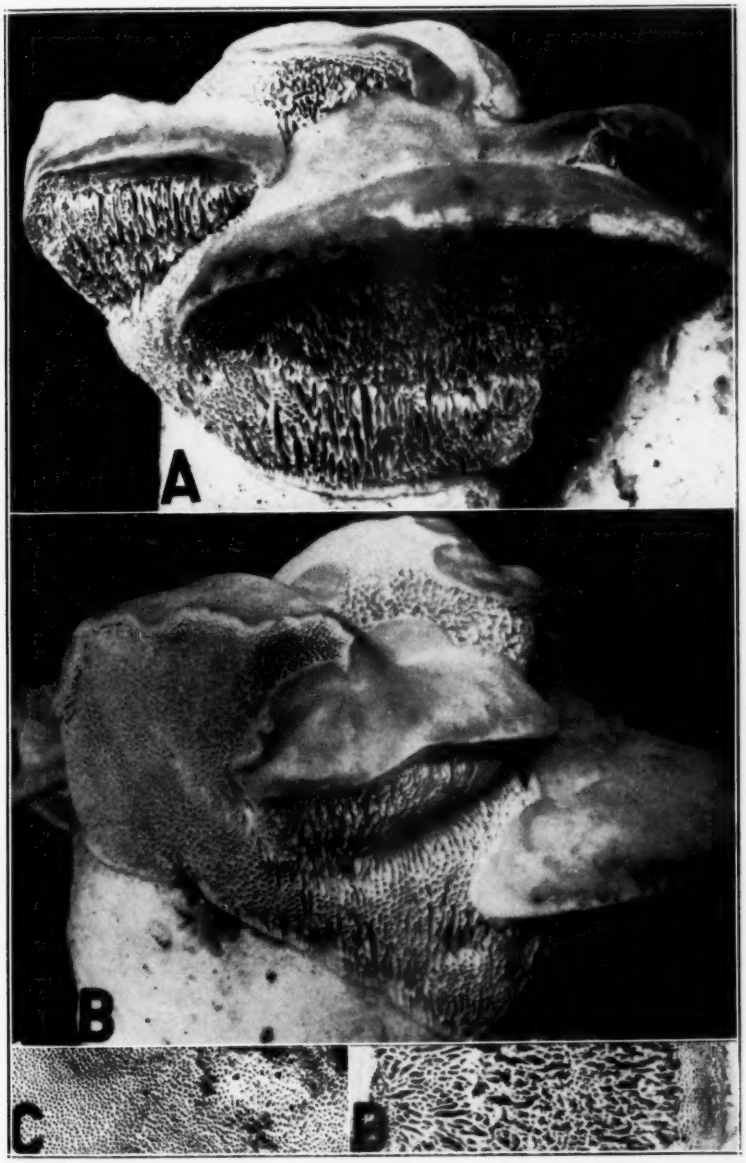

FIG. 1. A and B. Two views of the same clump of fruit bodies that developed on inoculated wood; note the horizontal pores. C. Pore surface of original fruit body. D. Pore surface of fruit body illustrated in A.

of both pieces was thoroughly rotted, but the grain also was almost entirely consumed. It would seem not an unreasonable assumption that the more

or less uniform diffused light may have played a part, since Christensen[2] reported a somewhat similar anomalous development of sporophores of *Daedalia confragosa* in diffused light in the laboratory. The fact that comparatively small differences in the environment can induce considerable variations in gross morphology of sporophores should make one wary of ascribing too much importance to minor morphologic differences between fruit bodies found in nature.—ELLIS F. DARLEY and CLYDE M. CHRISTENSEN, University Farm, St. Paul, Minn.

A Method for Maintaining Phytomonas sepedonica in Culture for Long Periods Without Transfer.—Phytomonas sepedonica, the organism causing ring rot of potato, may be considered a fastidious species and difficult to maintain on culture media. Even with Burkholder's special media no claims for longevity have been made. Stapp[1] failed to maintain it under any circumstances, even with repeated transfers for more than 18 months. The writer has found viability uncertain in transfers from agar cultures older than 30 days, kept under laboratory conditions.

Lumière and Chevrotier[2] maintained gonococci cultures several months when sealed with paraffin oil or vaseline. Michael,[3] employing solid media, extended the test to other organisms with good results. Bruni,[4] Parish,[5] and Birkhaug[6] kept a number of human bacterial pathogens viable under liquid paraffin for 8–24 weeks. These studies suggested the possibility of maintaining plant pathogens such as *Phytomonas sepedonica* under mineral oil; subsequent preliminary tests by this department indicated that the viability of the organism could be prolonged in this way. Approximately 2 years ago a number of fresh isolates of *P. sepedonica* were placed under sterile mineral oil and have since been tested for viability and pathogenicity.

Slants of Burkholder's media were inoculated with 16 cultures of *Phytomonas sepedonica*. Ten days later the cultures were covered with a layer of sterile mineral oil sufficient to extend well above the slant. Tubes were plugged with cotton and held at room temperature. To test viability at monthly intervals a 2-mm. loop was passed through the oil, a loopful of cells removed and streaked on a fresh agar slant. Before streaking, the loop was held against the inside wall of the test tube above the agar to remove much of the oil adhering to the cells. An effort was made to employ

[2] Christensen, C. M. Two cases of unusual development of fruit bodies. Mycologia **34**: 400–402. 1942.

[1] Stapp, C. Beiträge zur Kenntnis des *Bacterium sepedonicum* Spiekerm. et Kotth. des Erregers der "Bakteriumringfaul" der Kartoffel. Zeitschr. f. Parasitenk. **2**: 756–823. 1930.

[2] Lumière, A., and A. Chevrotier. Sur la vitalité des cultures de gonocoques. Comp. Rend. Acad. Sci. **158**: 1820–1821. 1914.

[3] Michael, M. Die Konservierung schwer. haltbarer Bakterienkulturen insbesondere des Gonococcus. Centralbl. f. Bakt. O. I. **86**: 507–510. 1921.

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[5] Parish, H. J. Preservation of cultures under liquid paraffin. Jour. Path. Bact. **35**: 143–144. 1932.

[6] Birkhaug, K. E. Preservation of bacterial cultures under liquid paraffin. Science (n.s.) **76**: 236–237. 1932.

a uniform technique for each culture and each test. Readings were made after 14 days incubation.

Viability of cultures of *P. sepedonica* for the duration of this test and the age at which viability was apparently lost are indicated in Table 1.

Series B differs from Series A only in being placed under oil 3 months earlier than the latter. All were colony isolates from diseased tubers. After 10 months under oil most cultures were still viable and grew luxuriantly on removal from oil. After 18 months 25 per cent of the cultures were alive. In Series B a few were still viable after 17 months; however, after 21 months all were dead. The difference between individual cultures in ability to exist under oil was noticeable, certain ones losing viability after 6, others

TABLE 1.—*Summary of viability of Phytomonas sepedonica under mineral oil*

Series A

	Number of months under oil								
	2	4	5	6	7	10	11	14	18
Total cultures tested	10	10	10	6[a]	6[a]	16[b]	16	16	16
Total cultures viable	10	9	9	5	5	10	8	4	4
Percentage of cultures viable	100	90	90	83	83	62.5	50	25	25

Series B

	Number of months under oil					
	6	8	13	14	17	21
Total cultures tested	10	10	8[a]	8	8	8
Total cultures viable	6	4	3	2	2	0
Percentage of cultures viable	60	40	37.5	25	25	0

[a] Some cultures lost by fungus contamination of oil.
[b] 10 additional cultures placed under oil at the same time as others but not previously tested for viability.

after 10, and some after 17 months. Cultures of *P. sepedonica*, viable after 18 months immersion, were tested for pathogenicity and induced typical ring-rot symptoms on leaf and tuber. Similar cultures without an oil covering will dry up and lose viability in 30–60 days.

Supplementary investigations employing other plant pathogens have demonstrated that *Phytomonas medicaginis* var. *phaseolicola* (halo blight of bean), and *P. phaseoli* (common blight of bean), under oil, retain their viability and pathogenicity at least 13–18 months. A like oil-immersion test applied to plant-pathogenic fungi indicates that the following remained apparently unharmed for at least 6 months (extent of test) and produced normal spore forms and mycelium when removed: *Fusarium eumartii, F. oxysporum, F. avenaceum, F. lycopersici,* and *Alternaria* sp. Further tests will be made at monthly intervals on all fungal and bacterial cultures to determine the viability end point.

It is believed this investigation has demonstrated the practicability of employing mineral oil for maintaining *Phytomonas sepedonica* and other phytopathogens for relatively long periods without transfer to new media.—ARDEN F. SHERF, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska.

On the Value of Spergon for Seed Treatment in Small-Grain Crops.—Among organic compounds recently found valuable for seed treatment, tetrachloro-para-benzoquinone, main constituent of the dust fungicide Spergon, is of special interest. Its slight toxicity for animals and its low phytocidal action, compared with many other chemicals, are combined properties of considerable merit. This dust, first employed as a seed protectant for Lima beans and garden peas[1,2] promises a wider field of usefulness.[3,4] To determine its possible value as a fungicide for small-grain seeds, field tests were undertaken in Alberta in 1941. A few of the results of these tests are here reported.

TABLE 1.—*Relative effectiveness of Spergon, Ceresan, and formaldehyde in the control of covered smuts of wheat and oats*

Treatment	Amount or concentration applied	Percentage smutted heads						
		Edmonton		Fallis		Olds		Castor
		Wheat	Oats	Wheat	Oats	Wheat	Oats	Wheat
Check	None	30.5	17.7	15.0	11.0	25.2	10.8	42.5
Ceresan	½ oz. per bu.	2.2	0.0	2.2	0.0	0.2	0.2	2.0
Spergon	2 oz. per bu.	0.3	7.0	0.0	1.2	1.0	11.5	0.2
Formaldehyde	1–320	0.0	0.2	0.0	0.0	0.0	0.5	0.0

Seed of wheat, oats, and flax was treated. The seed lots of the first two were artificially infested with their respective covered smut fungi and also carried a small amount of natural inoculum. In the case of flax, clean seed was used. Spergon was applied at the rate of 2 oz. per bu., a week before sowing. The other fungicides, tested comparatively, were applied according to usual recommendations at the rates indicated in the tables. Tests with flax were made at Edmonton and Castor, Alberta; the other grains were tested at Fallis and Olds, as well. At Edmonton, 6, elsewhere 4, replicates of each treatment and each check, randomized in blocks, were sown. The locations mentioned represent different soil types and climatic conditions. Castor is located in the dry open prairie in the brown-soil belt, Edmonton and Olds are in areas of higher rainfall in the black-soil park country, and Fallis in a wooded, gray-soil region. The same seed samples were used at all points.

[1] Cunningham, H. S., and E. G. Sharvelle. Organic seed protectants for Lima beans. Phytopath. (Abstract) **30**: 4–5. 1940.

[2] Sharvelle, E. G., and B. F. Shema. A preliminary investigation of the value of a new seed protectant for canning peas in Minnesota. Phytopath (Abstract) **31**: 20. 1941.

[3] Felix, E. L. Tetrachloro-para-benzoquinone, an effective organic seed protectant Phytopath. (Abstract) **32**: 4. 1942.

[4] Leukel, R. W. Spergon as a seed disinfectant. Pl. Dis. Rptr. **26**: 93–94. 1942.

Data on covered-smut control in wheat and oats are shown in table 1. The figures are averages of the replicates of each treatment. It will be noted that Spergon gave good control of covered smut or bunt of wheat at all points, comparing favorably in effectiveness with Ceresan and formaldehyde. In the case of oats, however, Spergon did not control covered smut as well as either Ceresan or formaldehyde.

The relative effects of Spergon and Ceresan on flax are indicated by average emergence and yield figures (Table 2). Both fungicides improved

TABLE 2.—*Relative effects of Spergon and Ceresan on the emergence and yield of flax*

Treatment	Amount applied	Edmonton		Castor	
		Emergence	Yield	Emergence	Yield
	Oz.	*Per cent*	*Bu. per a.*	*Per cent*	*Bu. per a.*
Check	None	53.0	8.4	59.8	5.5
Ceresan	0.5	81.5	9.3	71.1	6.6
Spergon	2.0	75.3	10.2	65.1	6.4

the emergence significantly at Edmonton, and Ceresan proved superior to Spergon (M.S.D. = 6). At Castor, a similar relationship held, though the difference between the fungicides was not significant. A tendency for both fungicides to improve yield is perhaps indicated by the consistently higher figures for the treated over the untreated seed lots. The differences between checks and treatments and between the treatments are, however, not significant. While yield response may be determined in part by emergence response, other factors may be even more important.

It may be concluded that Spergon has merit as a seed fungicide, especially for wheat and flax.—A. W. HENRY, University of Alberta, Edmonton, Alberta, Canada.

Alternaria sp. on Grain Kernels Killed by High Temperature Storage.—In connection with studies in 1941 on the effects of storage temperatures on germinability of certain seeds, it was observed that those of oats, wheat, and barley, stored 6 to 15 months at 105° F. were free from fungi; those from the same seed lots, stored at 36° and 50° F., were heavily infested, chiefly with *Alternaria* sp. Oat kernels stored 15 months at 36° F. (Fig. 1, A), wheat 6 months at 50° F., and barley 7 months at 36° F. (Fig. 1, E), developed abundant growth of *Alternaria* sp. during the regular seed-germination period of from 3 to 7 days. On kernels of oats, wheat, and barley from the same lots of seeds, stored for the same periods at 105° F., the *Alternaria* sp. had been killed and, on germination, the seeds were free from the fungus (Fig. 1, B, F). All the seeds were germinated on moist blotters in germinating dishes.

The seeds on which the above observations were made were received as follows: Oats from Aberdeen, Idaho, August 16, 1939; wheat from Lincoln,

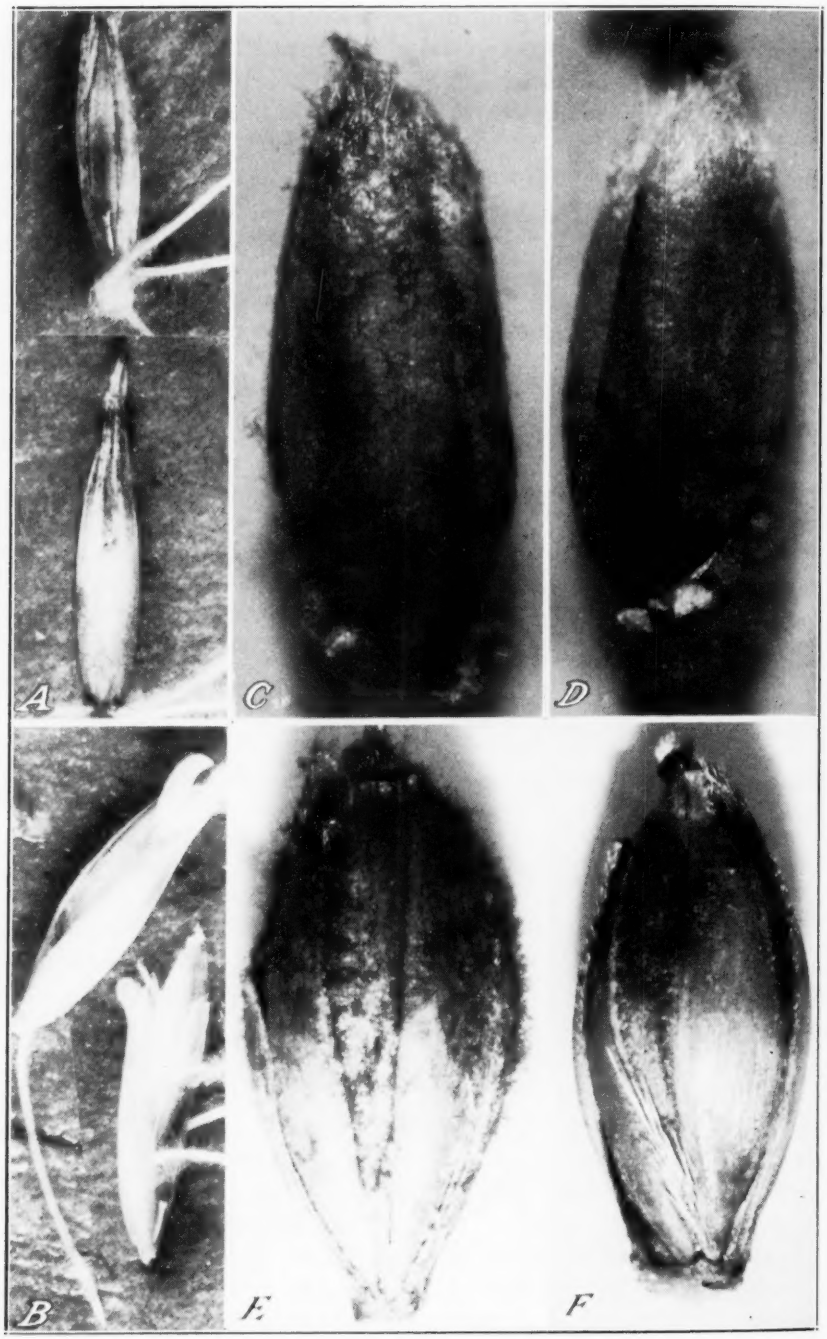

FIG. 1. A and B. Kernels of oats stored 15 months: A, at 36° F.; B, at 105°. C and D. Wheat stored 7 months: C, at 50°; D, at 105°. E and F. Barley stored 7 months: E, at 36°; F, at 105°. All germinated on moist blotters in germinating dishes. A, C, and E, stored at the lower temperatures, showed abundant development of *Alternaria* sp.; B, D, and F, stored at 105° F., were free from the fungus. In C to F, the primary roots were clipped off.

Nebraska, July 2, 1940; and barley from Arlington Farm, Virginia, June 21, 1940. In each case when the grains had been stored 6 months or longer at 105° F., the fungus originally on the kernels was killed; in the samples stored at 36° and 50° F., the fungus was not killed.

A test was made to confirm the above observation. The seed used was wheat received from Lincoln, Nebraska, July 2, 1940. One sample of the wheat had been stored 7 months at 50° F. and another for the same period at 105° F. Eight kernels from each sample were placed on moist blotters in germinating dishes under sterile conditions. After 7 days incubation at 68° F., the kernels, all of which had germinated, were examined for the presence of *Alternaria* sp. The wheat kernels that had been stored 7 months at 50° F. were heavily infected with *Alternaria* sp.[1] (Fig. 1, C), while those stored for the same period at 105° F. were free from the fungus (Fig. 1, D). —EDGAR BROWN AND ALICE L. ROBERT, Bureau of Plant Industry, U. S. Dept. of Agr., Washington, D. C.

Stem Rust on Triticum timopheevi.[1]—*Triticum timopheevi* Zhuk. has been highly resistant to practically all physiologic races of stem rust, *Puccinia graminis tritici*, with which it has been inoculated. But race 189, an unusually virulent race in Peru, can attack seedlings and adult plants of *T. timopheevi*;[2] and race 19, identified more often than any other race in collections from experimental field plantings of *T. timopheevi* in the United States, has been moderately virulent on seedlings and on older plants inoculated in the greenhouse by the writer and by Stakman and his co-workers.

In 1942 there was 35 per cent of stem rust on *Triticum timopheevi* in certain experimental plots at University Farm, St. Paul, Minnesota. All plant parts were rusted, from the lowermost leaf sheaths to the glumes, awns, and peduncles. The reaction indicated moderate susceptibility, although most pustules were small and surrounding tissues were slightly discolored (Fig. 1, A). Wheat varieties and hybrids in the experimental plots were exposed to stem-rust inoculum early in the season, border rows between plots being inoculated as early as May 26, by means of a hypodermic syringe, with water suspensions of urediospores of six different physiologic races (15B, 17, 34, 36, 56, 147) of *P. graminis tritici*. By June 3 inoculum was spreading from rusted borders to the experimental plants. Inoculations every 5 to 7 days provided a new and an ever-increasing supply of rust in the borders for a natural spread to the experimental plants, which themselves never were inoculated directly.

Seven collections of stem rust were taken from various replicates in the

[1] The *Alternaria* on the wheat kernels was identified as belonging to the *A. tenuis* group, as interpreted by Elliott, J. A., Am. Jour. Bot. 4: 472. 1917.

[1] Published as Paper 2039 in the Journal Series of the Minnesota Agricultural Experiment Station. Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project No. 265–1–71–236, Subproject No. 491.

[2] Garcia-Rada, G., J. Vallega, W. Q. Loegering, and E. C. Stakman. An unusually virulent race of wheat stem rust, no. 189. Phytopath. 32: 720–726. 1942.

3 different plantings of *Triticum timopheevi*. Upon identification every collection consisted of race 15B, apparently the only one of the 6 races furnished in the inoculum that was virulent on this species of *Triticum*. Newton, Johnson, and Peterson[3] reported on the seedling reaction of *T. timopheevi* to 20 physiologic races of *Puccinia graminis tritici*, among which was race 15. The species was highly resistant and from the work of Stakman and Loegering[4] it seems that the Canadian race 15 was different from the race

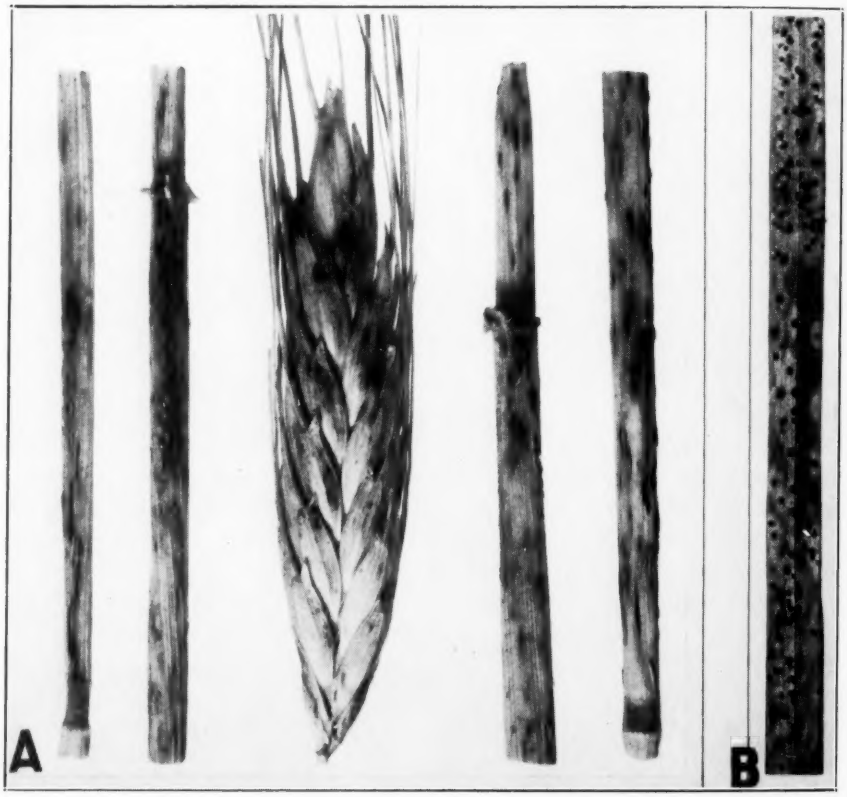

FIG. 1. Stem rust on *Triticum timopheevi* Zhuk. A. Telia of stem rust on culms and head of *T. timopheevi* grown in the field at St. Paul in 1942. The stems were boiled in sodium hypochlorite before they were photographed, and most of the hairs that characterize the host species were broken. ×about 2. B. The susceptible reaction of seedlings of *T. timopheevi* to race 15B of *Puccinia graminis tritici*. ×about 2.

15B used in the present work. Seedlings of *T. timopheevi* were susceptible to race 15B and gave a type 3+ reaction (Fig. 1, B). Loegering and Stakman[4] reported that race 15B is virulent on Rival, a newly recommended variety of hard red spring wheat, whereas race 15A does not attack it. They pointed out the danger of such a biotype of race 15 in the spring wheat area

[3] Newton, Margaret, T. Johnson, and B. Peterson. Seedling reactions of wheat varieties to stem rust and crown rust. Can. Jour. Res. (C) 18: 489–506. 1940.

[4] Loegering, W. Q., and E. C. Stakman. Biotypes within *Puccinia graminis tritici*, race 15. (Abstract) Phytopath. 32: 12–13. 1942.

and found that both types of the race are present in the United States. It now seems probable that *Triticum timopheevi* and its hybrids also will be endangered by race 15B if that race of rust becomes widespread and destructive in wheat-growing areas and if inoculum is abundant and conditions favor rust infection for a protracted period. This is merely another illustration of the fact that there are few species of wheat or wheat allies that are universally resistant to rust.—HELEN HART, Agricultural Experiment Station, University Farm, St. Paul, Minn.

BOOK REVIEW

BALDWIN, HENRY IVES. *Forest Tree Seed.* Chronica Botanica Co., Waltham, Mass.; G. E. Steehert and Co., New York City. 240 pp. 28 figs., 1942. $4.75.

The author has selected and compiled considerable technical and practical knowledge on tree seed. Although the book is of value to all engaged in handling various kinds of seed and studying different aspects of the whole field of seed problems, "Forest Tree Seed" will be most helpful to silviculturists and especially so to those interested in the conservation and reforestation of timberlands.

The subject matter is divided into 20 chapters. The information presented centers around such topics as development, structure, and production of tree seed; some of the other phases of the seed problems that are presented include origin, collection, extraction, and germination of seed, internal and environmental factors affecting germination, and seed viability and stimulation. Chapter 7, Biotic enemies of tree seeds, contains some sections especially interesting to the pathologist. They are as follows: Fungi, Seed-borne diseases, Diseases of cones, Seed disinfection and antisepsis, Physical treatments, Chemical treatments, and Aseptic germination. Each topic is developed by a résumé of most of the pertinent literature with significant specific details being given in small type. Reference lists, most of which are extensive, are to be found at the end of all but the last chapter, which consists of a glossary of tree-seed terms and subject and author indices.

The outstanding contribution of the book is that it brings together and makes readily available a considerable amount of knowledge heretofore to be found only in unassembled publications.—WILLIAM C. DAVIS, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

WAR COMMITTEE OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY—REPORT OF MEETING IN COLUMBUS, OHIO, FEBRUARY 13 AND 14, 1943

The committee met in conjunction with a meeting of the officers and the Council of the Society called in place of the cancelled annual meeting. The Plant Protection Committee of the National Research Council met with the War Committee. E. F. Phillips, chairman of the War Committee of the Association of Economic Entomologists, and Neil Stevens, chairman of the War Committee of the American Mycological Society, were present as representatives of their respective committees. The following members of the War Committee were present: C. C. Allison, G. M. Armstrong, H. P. Barss, F. J. Greaney, R. J. Haskell, J. G. Horsfall, L. M. Hutchins, R. S. Kirby, I. E. Melhus, J. C. Walker, and J. G. Leach (Acting Chairman in the absence of E. C. Stakman).

All day Saturday was spent hearing and discussing the reports of regional committees, sub-committees, and members at large. At a final meeting held late Sunday afternoon, a number of resolutions were adopted. These appear at the end of this report.

Space will not permit a complete report of Saturday's meeting. A more detailed report has been mimeographed and sent to the contact men in each State. Some of the more significant items reported were as follows:

Attention was called to occupational Bulletin No. 23 as amended December 14, 1942, authorizing draft boards to defer not only full-time instructors and research workers in agricultural sciences, but also part-time research assistants if working on projects certified to be related to the war effort.

Local Board Release No. 159, authorizing the establishment of special committees on manpower in critical scientific fields, was discussed. The Executive Committee has requested G. W. Keitt to make a survey of the situation in Plant Pathology to determine if such a committee is desirable. *It was voted to defer submission of the resolution on manpower, drawn up by the Upper Mississippi Valley Group at its Chicago meeting, until after this survey is completed.*

A memorandum, prepared by E. C. Stakman, and submitted to Governor Lehman, Director of the Office of Foreign Relief and Rehabilitation Operations, by Ross G. Harrison, Chairman of the National Research Council, was explained briefly. This memorandum recommends that people in the countries occupied by American armed forces be helped to help themselves through increased efficiency in agricultural production. It is further recommended that men with specialized training in agricultural sciences, already in the armed forces, be detailed by the Army to cooperate with other authorities in the direction of such a program.

The importance of regional meetings to consider problems of mutual interest in the war effort was next discussed. In view of some difficulties already experienced by workers in some States in receiving authorization to attend regional meetings of the War Committee, *the Executive Committee was instructed to write a suitable letter to the Directors of Experiment Stations and Directors of Agricultural Extension, soliciting their interest in, and encouragement of, regional meetings during the war period.*

Dr. Stakman's recent suggestion that quarterly reports of the War Committee be published in Phytopathology was discussed and approved by the Committee.

A suggestion that the name of the Committee be modified by eliminating the word "Emergency" was approved.

F. J. Greaney told of the manpower situation in Canada, where all universities are under complete military control, and where plant pathologists are being called upon for medical aid on the home front.

J. C. Walker reported on a program of bean-seed certification for control of bacterial blight, being developed by L. H. Person of Louisiana. He also stressed the critical shortage of many vegetable seeds and the importance of seed-treatment in seed conservation.

E. F. Phillips reported on manpower situation in Entomology, pointing out that the Army is making good use of the technical training of entomologists, practically all entomologists inducted into the service being assigned to duty as entomologists, but that the demand of the Army for entomologists is increasing steadily, and has already reached the point where the effectiveness of entomology in the food-production plan is being threatened.

J. G. Horsfall reported briefly on coordinated research on fungicide dosage and substitute fungicides. More complete reports will be mimeographed and distributed.

R. J. Haskell reminded the committee of his offer to distribute informational material to 130 contact men through the country, and stressed the importance of more adequate exchange of information.

Harry O'Brien led an inspiring discussion on problems of publicity. He gave us many valuable suggestions and promised to help us as much as possible.

At the final meeting, called at 3:00 o'clock, Sunday afternoon, the following resolutions were adopted:

1. That a nation-wide seed-treatment campaign be declared for the reduction of plant-disease losses of the crops essential in the war-production program with special emphasis on oats, barley, wheat, sorghum, flax, corn, cotton, peanuts, and certain vegetable crops as a means of insuring against poor stands and as a means of stretching limited seed supplies.

2. That efforts be made to include established plant-disease-control recommendations into the action programs for achieving war-crop goals.

3. That plant pathologists in each State be urged to make every effort to obtain information on the development of major disease hazards throughout the season, with the aid of such other agencies and individuals as may be available, with a view to the issuance of timely warnings to growers and recommendations of immediate measures to be taken to check epidemics, or to lessen losses that would otherwise result.

4. That the Seed-treatment Committee of the Society be asked to prepare 1943 recommendations for the seed-treatmnet of wheat, oats, barley, sorghum, flax, and corn for distribution to all States.

5. That the Seed-treatment Committee be encouraged to work with seed producers and seedsmen with a view toward getting more seeds treated prior to distribution and sale, and/or getting directions for treatment printed on packages, or packets and in seed catalogs.

6. That efforts of the Seed Certification Committee to obtain the treatment of certified seed be endorsed by the War Committee.

7. That the Fungicide Committee of the War Committee be asked to distribute promptly a condensed statement of their present opinions as to measures for conserving fungicides.

Respectfully submitted,
J. G. LEACH
Acting Chairman